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(54) Title: CUTINASE VARIANTS

(57) Abstract

Variants of fungal cutinases have improved thermostability. The variants comprise substitution of one or more amino acid residues near the N–terminal in the amino acid sequence or in the three–dimensional structure of the cutinase.

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CUTINASE VARIANTS

FIELD OF THE INVENTION

The present invention relates to a cutinase variant, more particularly to a cutinase variant having improved thermostability. The invention also relates to a DNA sequence encoding the variant, a vector comprising the DNA sequence, a transformed host cell harboring the DNA sequence or the vector, to a method of producing the variant, and to use of the variant.

BACKGROUND OF THE INVENTION

Cutinases are lipolytic enzymes capable of hydrolyzing the substrate cutin. Cutinases are known from various fungi (P.E. Kolattukudy in "Lipases", Ed. B. Borgström and H.L. Brockman, Elsevier 1984, 471-504). The amino acid sequence and the crystal structure of a cutinase of *Fusarium solani pisi* have been described (S. Longhi et al., Journal of Molecular Biology, 268 (4), 779-799 (1997)). The amino acid sequence of a cutinase from *Humicola insolens* has also been published (US 5,827,719).

A number of variants of the cutinase of *Fusarium solani pisi* have been published: WO 94/14963; WO 94/14964; Appl. Environm. Microbiol. 64, 2794-2799, 1998; Proteins: Structure, Function and Genetics 26, 442-458, 1996; J. of Computational Chemistry 17, 1783-1803, 1996; Protein Engineering 6, 157-165, 1993; Proteins: Structure, Function, and Genetics 33, 253-264, 1998; J. of Biotechnology 66, 11-26, 1998; Biochemistry 35, 398-410, 1996.

Fungal cutinases may be used in the enzymatic hydrolysis of cyclic oligomers of poly(ethylene terephthalate), e.g. in the finishing of yarn or fabric from poly(ethylene terephthalate) fibers (WO 97/27237). However, it is desirable to improve the thermostability of known fungal cutinases to allow a higher process temperature.

SUMMARY OF THE INVENTION

The inventors have found certain variants of fungal cutinases having improved thermostability.

Accordingly, the invention provides a variant of a parent fungal cutinase comprising substitution of one or more amino acid residues which is located:

a) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

b) within 20 positions from the N-terminal amino acid.

The invention also provides a DNA sequence encoding the variant, an expression vector comprising the DNA sequence, a transformed host cell harboring the DNA sequence or the expression vector, a method of producing the variant, processes using the variant and a detergent composition comprising the variant.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 gives the coordinates for the 3D structure of the cutinase of *H. insolens*.

Fig. 2 is a computer model showing the three-dimensional structures of the cutinases from *F. solani pisi* (left) and *H. insolens* (right). Different colors have been used to identify the N-terminal amino acid and zones of 12 Å and 17 Å diameter around this.

Figs. 3-6 illustrate the hydrolysis of c3ET. Details are given in the Examples.

DETAILED DESCRIPTION OF THE INVENTION

Fungal cutinase

The parent cutinase is a fungal cutinase, such as a filamentous fungal cutinase, e.g. native to a strain of *Humicola* or *Fusarium*, specifically *H. insolens* or *F. solani pisi*, more specifically *H. insolens* strain DSM 1800.

The amino acid sequence of the cutinase of *H. insolens* strain DSM 1800 and the DNA sequence encoding it are shown as SEQ ID NO: 2 and SEQ ID NO: 1 of US 5,827,719. The numbering system used herein for the *H. insolens* cutinase is based on the mature peptide, as shown in said SEQ ID NO: 2.

The amino acid sequence of the cutinase of *F. solani pisi* is shown as the mature peptide in Fig. 1D of WO 94/14964. The numbering system used herein for

the *F. solani pisi* cutinase is that used in WO 94/14964; it includes the pro-sequence shown in said Fig. 1D; thus, the mature cutinase is at positions 16-214.

The parent cutinase may have an amino acid sequence which is at least 50 % (particularly at least 70 % or at least 80 %) homologous to the cutinase of *H. insolens* strain DSM 1800. The parent cutinase may particularly be one that can be aligned with the cutinase of *H. insolens* strain DSM 1800.

Nomenclature for amino acids and alterations

The specification and claims refer to amino acids by their one-letter codes. A particular amino acid in a sequence is identified by its one-letter code and its position, e.g. Q1 indicates Gln (glutamine at position 1, i.e. at the N-terminal.

The nomenclature used herein for defining substitutions is basically as described in WO 92/05249. Thus, R51P indicates substitution of R (Arg) at position 51 with P (Pro).

Homology and alignment

For purposes of the present invention, the degree of homology may be suitably determined according to the method described in Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-45, with the following settings for polypeptide sequence comparison: GAP creation penalty of 3.0 and GAP extension penalty of 0.1. The determination may be done by means of a computer program known such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711).

Two given sequences can be aligned according to the method described in Needleman (*supra*) using the same parameters. This may be done by means of the GAP program (*supra*).

Three-dimensional structure of cutinase

The structure of the cutinase of *H. insolens* was solved in accordance with the principle for X-ray crystallographic methods as given, for example, in X-Ray Structure Determination, Stout, G.K. and Jensen, L.H., John Wiley & Sons, Inc. NY, 1989. The structural coordinates for the solved crystal structure at 2.2 Å resolution

using the isomorphous replacement method are given in Fig. 1 in standard PDB format (Protein Data Bank, Brookhaven National Laboratory, Brookhaven, CT).

The structure of the cutinase of *F. solani pisi* is described in Martinez et al. (1992) Nature 356, 615-618. The 3D structures of the cutinases of *F. solani pisi* and *H. insolens* are compared as a computer model in Fig. 2.

It should be noted that the overall three-dimensional structures of fungal cutinases are very similar and have been shown by X-ray crystallography to be highly homologous. The similarities between the cutinases from *F. solani pisi* and *H. insolens* are clearly apparent from the computer model in Fig. 2. Therefore, modifications of the type indicated for one fungal cutinase will also be functional for other fungal cutinases.

Substitution near N-terminal

The variant of the invention has one or more amino acid substitutions in the vicinity of the N-terminal. The substitution is within a distance of 17 Å (e.g. within 12 Å) and/or within 20 positions (e.g. within 15 positions) of the N-terminal. The distance from the N-terminal is to be calculated between the Cα atom of the amino acids, and is calculated from an amino acid in a crystal structure (i.e. visible in the X-ray structure).

In the cutinase of *H. insolens* strain DSM 1800, the two N-terminal amino acids (Q1 and L2. i.e. Gln and Leu at positions 1 and 2) are not visible in the X-ray structure, so the distance is to be calculated from amino acid G3. Amino acids within 17 Å include positions 3-12, 18, 20-60, 62-64, 82, 85-86, 100-108, 110-111, 130-132, 174, 176-182, 184-185, 188, and 192. Those within 12 Å include positions 3-8, 22-27, 30-47, 53-59, 102, 177, and 180-181.

In the cutinase of *F. solani pisi*, the N-terminal amino acid G17 is visible in the X-ray structure. Amino acids within 17 Å include positions 17-26, 34-75, 77-79, 101, 115, 117-119, 147, 191-197, 199-200, and 203. Those within 12 Å include positions 17-22, 38, 40, 45-58, 60, 65, and 70-72.

Th variants of the invention have improved thermostability compared to the par nt enzyme. The thermostability may be determined from the denaturation tem-

perature by DSC (differential scanning calorimetry), e.g. as described in an example, e.g. at pH 8.5 with a scan rate of 90 K/hr. The variants may have a denaturation temperature which is at least 5°C higher than the parent enzyme.

The total number of substitutions in the above regions is typically 1-10, e.g. 1-5 substitutions in the above regions. In addition, the cutinase variant of the invention may optionally include other modifications of the parent enzyme, typically 10 or fewer, e.g. 5 or fewer alterations (substitutions, deletions or insertions) outside of the above regions. Thus, the total amino acid sequence of the variant typically 1-20, e.g. 1-10 alterations compared to the parent cutinase.

Solvent accessible surface

One or more of the substitutions may be made at an exposed amino acid residue, i.e. an amino acid residue having a solvent accessible surface. This can be calculated by the "dssp" program (version October 1988) described in W. Kabsch and C. Sander, Biopolymers, 22 (1983) pp. 2577-2637.

In the cutinase of *H. insolens* strain DSM 1800, the following amino acids lie within 17 Å of G3 at the N-terminal and have a solvent accessible surface greater than 0: 3-12, 18, 26-33, 36-38, 40-45, 47-56, 59-60, 62-64, 82, 85-86, 104-105, 174, 176-179, 181-182, 192.

Specific substitutions

The substitution near the N-terminal may specifically be one that increases the electrical charge, i.e. a substitution of a negatively charged amino acid with a neutral or positively charged amino acid or substitution of a neutral amino acid with a positively charged amino acid. Thus, a negative amino acid residue at a position corresponding to position E6, E10, E30, E47 D63, E82 and/or E179 in the cutinase of *Humicola insolens* strain DSM 1800 may be substituted by a neutral or positive amino acid, e.g. R, K, Y, H, Q or N. Some specific substitutions are those corresponding to E6Q/N, E10Q/N, E47K/R or E179Q/N. Also, a neutral amino acid residue at a position corresponding to N7, S11, N44 or N52 in the *H. insolens* cutinase may be substituted by a positive amino acid (R, K or H).

Another example of a substitution near the N-terminal is substitution with a Pro residue, e.g. a substitution corresponding to A14P or R51P in the cutinase of *Humicola insolens* strain DSM 1800.

Specific variants

The following are some examples of variants in the *H. insolens* cutinase. Corresponding variants may be made on the basis of other parent cutinases.

R51P

E6N/Q+ L138I

A14P+ E47K

E47K

E179N/Q

E6N/Q+ E47K+ R51P

A14P+ E47K+ E179N/Q

E47K+ E179N/Q

E47K+ D63N

E6N/Q+ E10N/Q+ A14P+ E47K+ R51P+ E179N/Q

E6N/Q+ A14P+ E47K+ R51P+ E179N/Q

Q1P+ L2V+ S11C+ N15T+ F24Y+ L46I+ E47K

Use of cutinase variant

The cutinase variant of the invention may be used, e.g., for the enzymatic hydrolysis of cyclic oligomers of poly(ethylene terephthalate), such as cyclic tri(ethylene terephthalate), abbreviated as c3ET.

In particular, this may be used to remove such cyclic oligomers from polyester containing fabric or yarn by treating the fabric or yarn with the cutinase variant, optionally followed by rinsing the fabric or yarn with an aqueous solution having a pH in the range of from about pH 7 to about pH 11. The treatment of polyester is conveniently carried out above the glass transition temperature of c3ET (about 55°C) and below the glass transition temperature of polyester (about 70°C). Thus, the treatment may suitably be carried out at 50-80°C, e.g. at 60-75°C. The process may be carried out in analogy with WO 97/27237.

The cutinase variant may be used to treat polyester-containing textile. e.g. PET (polymer of ethyleneglycol and terephthalic acid), P3GT (polymer of 1,3-propanediol and terephthalic acid) or a polyester/cotton blend. The treatment may provide benefits to the polyester textile such as improved wear and comfort, increased water permeability, reduced antistatic behavior, improve handle and softness, changed redeposition characteristics and/or color clarification.

The cutinase variant may be used to improve the functional finish of a PET-containing yarn or fabric by a treatment with the cutinase variant, followed by a treatment with a finishing agent such as a softener, an anti-crease resin, an anti-static agent, an anti-soiling agent or agents to impair wrinkle-free, permanent press ior fire resistance effects. The treatment with the cutinase variant may increase the number of functional groups in the surface, and this can be used to attach the functional finish. Examples of finishing agents are described in "SENSHOKU SIAGEKAKO BENRAN" published 1998-10-15 by Nihon Seni Sentaa KK.

The cutinase variant of the invention is also useful in detergents, where it may be incorporated to improve the removal of fatty soiling, as described in WO 94/03578 and WO 94/14964. The addition of the cutinase variant to laundry detergent may reduce malodor from cloth which is accumulated during several wash/wear-cycles.

The cutinase variant may also be used for degradation and recycling of polyester such as polycaprolactone (PCL), poly-ethyleneglycol-terephthalate (PET), polylactic acid, polybutylenesuccinate, and poly(hydroxybutiric acid)-co-(hydroxyvaleric acid), e.g. film and bottles, e.g. as described in JP-A 5-344897.

The cutinase variant may also be used for other known applications of lipases and cutinases, for example, in the baking industry (e.g. as described in WO 94/04035 and EP 585988), in the papermaking industry (e.g. for pitch removal, see EP 374700), and in the leather, wool and related industries (e.g. for degreasing of animal hides, sheepskin or wool), and for other applications involving degreasing/defatting. It may be used in immobilized form in the fat and oil industry, as a catalyst in organic synthesis (e.g. sterification, transesterification or ester hydrolysis reactions).

Dyeing polyester

The invention provides a process for dyeing polyester fabric or yarn. In this process, the fabric or yarn is first treated with a cutinase, e.g. 12-48 hours at 50-70°C or 65-70°C, pH 7-10, followed by dyeing with dye, e.g. a reactive dye, a disperse dye or a cationic dye. The reactive dye may be one that reacts with OH or COOH groups, e.g. having the structure Chromophore-NHPh-$SO_2CH_2CH_2OSO_3Na$. The dyeing may be conducted at 40-80°C, e.g. for 20-60 minutes.

The cutinase may be a thermostable cutinase having a thermal denaturation temperature, T_d, at pH 8.5 which is at least 5° higher than the parent cutinase, e.g. 7-10° higher, e.g. a value of 65°C or higher. The measurement may be made by DSC as described in an Example of this specification.

Surfactant

In the treatment of fabric or yarn, a conventional wetting agent and/or a dispersing agent may be used to improve the contact with the enzyme. The wetting agent may be a nonionic surfactant, e.g. an ethoxylated fatty alcohol. A very useful wetting agent is an ethoxylated and propoxylated fatty acid ester such as Berol 087 (product of Akzo Nobel, Sweden).

The dispersing agent may suitably be selected from nonionic, anionic, cationic, ampholytic or zwitterionic surfactants. More specifically, the dispersing agent may be selected from carboxymethylcellulose, hydroxypropylcellulose, alkyl aryl sulfonates, long-chain alcohol sulfates (primary and secondary alkyl sulfates), sulfonated olefins, sulfated monoglycerides, sulfated ethers, sulfosuccinates, sulfonated methyl ethers, alkane sulfonates, phosphate esters, alkyl isothionates, acyl-sarcosides, alkyltaurides, fluorosurfactants, fatty alcohol and alkylphenol condensates, fatty acid condensates, condensates of ethylene oxide with an amine, condensates of ethylene oxide with an amide, sucrose esters, sorbitan esters, alkyloamides, fatty amine oxides, ethoxylated monoamines, ethoxylated diamines, alcohol ethoxylate and mixtures thereof. A very useful dispersing agent is an alcohol ethoxylate such as Berol 08 (product of Akzo Nobel, Sweden).

M thods for preparing cutinase variants

The cutinase variant of the invention can be prepared by methods known in the art, e.g. as described in WO 94/14963 or WO 94/14964 (Unilever). The following describes methods for the cloning of cutinase-encoding DNA sequences, followed by methods for generating mutations at specific sites within the cutinase-encoding sequence.

Cloning a DNA sequence encoding a cutinase

The DNA sequence encoding a parent cutinase may be isolated from any cell or microorganism producing the cutinase in question, using various methods well known in the art. First, a genomic DNA and/or cDNA library should be constructed using chromosomal DNA or messenger RNA from the organism that produces the cutinase to be studied. Then, if the amino acid sequence of the cutinase is known, labeled oligonucleotide probes may be synthesized and used to identify cutinase-encoding clones from a genomic library prepared from the organism in question. Alternatively, a labeled oligonucleotide probe containing sequences homologous to another known cutinase gene could be used as a probe to identify cutinase-encoding clones, using hybridization and washing conditions of lower stringency.

Yet another method for identifying cutinase-encoding clones would involve inserting fragments of genomic DNA into an expression vector, such as a plasmid, transforming cutinase-negative bacteria with the resulting genomic DNA library, and then plating the transformed bacteria onto agar containing a substrate for cutinase (*i.e.* maltose), thereby allowing clones expressing the cutinase to be identified.

Alternatively, the DNA sequence encoding the enzyme may be prepared synthetically by established standard methods, *e.g.* the phosphoroamidite method described S.L. Beaucage and M.H. Caruthers, (1981), Tetrahedron Letters 22, p. 1859-1869, or the method described by Matthes et al., (1984), EMBO J. 3, p. 801-805. In the phosphoroamidite method, oligonucleotides are synthesized, *e.g.* in an automatic DNA synth sizer, purifi d, annealed, ligated and cloned in appropriate vectors.

Finally, the DNA sequence may be of mixed genomic and synthetic origin, mixed synthetic and cDNA origin or mixed genomic and cDNA origin, prepared by

ligating fragments of synthetic, genomic or cDNA origin (as appropriate, the fragments corresponding to various parts of the entire DNA sequence), in accordance with standard techniques. The DNA sequence may also be prepared by polymerase chain reaction (PCR) using specific primers, for instance as described in US 4,683,202 or R.K. Saiki et al., (1988), Science 239, 1988, pp. 487-491.

Site-directed mutagenesis

Once a cutinase-encoding DNA sequence has been isolated, and desirable sites for mutation identified, mutations may be introduced using synthetic oligonucleotides. These oligonucleotides contain nucleotide sequences flanking the desired mutation sites. In a specific method, a single-stranded gap of DNA, the cutinase-encoding sequence, is created in a vector carrying the cutinase gene. Then the synthetic nucleotide, bearing the desired mutation, is annealed to a homologous portion of the single-stranded DNA. The remaining gap is then filled in with DNA polymerase I (Klenow fragment) and the construct is ligated using T4 ligase. A specific example of this method is described in Morinaga et al., (1984), Biotechnology 2, p. 646-639. US 4,760,025 discloses the introduction of oligonucleotides encoding multiple mutations by performing minor alterations of the cassette. However, an even greater variety of mutations can be introduced at any one time by the Morinaga method, because a multitude of oligonucleotides, of various lengths, can be introduced.

Another method for introducing mutations into cutinase-encoding DNA sequences is described in Nelson and Long, (1989), Analytical Biochemistry 180, p. 147-151. It involves the 3-step generation of a PCR fragment containing the desired mutation introduced by using a chemically synthesized DNA strand as one of the primers in the PCR reactions. From the PCR-generated fragment, a DNA fragment carrying the mutation may be isolated by cleavage with restriction endonucleases and reinserted into an expression plasmid.

Expr ssion of cutinas variants

According to the invention, a DNA sequence encoding the variant produced by methods described above, or by any alternative methods known in the art, can be expressed, in enzyme form, using an expression vector which typically includes con-

trol sequences encoding a promoter, operator, ribosome binding site, translation initiation signal, and, optionally, a repressor gene or various activator genes.

Expression vector

The recombinant expression vector carrying the DNA sequence encoding a cutinase variant of the invention may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. The vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated. Examples of suitable expression vectors include pMT838.

Promoter

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell.

Examples of suitable promoters for directing the transcription of the DNA sequence encoding a cutinase variant of the invention, especially in a bacterial host, are the promoter of the *lac* operon of *E.coli*, the *Streptomyces coelicolor* agarase gene *dag*A promoters, the promoters of the *Bacillus licheniformis* α-amylase gene (*amyL*), the promoters of the *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), the promoters of the *Bacillus amyloliquefaciens* α-amylase (*amyQ*), the promoters of the *Bacillus subtilis* xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding *A. oryzae* TAKA amylase, the TPI (triose phosphate isomerase) promoter from *S. cerevisiae* (Alber et al. (1982), J. Mol. Appl. Genet 1, p. 419-434, *Rhizomucor miehei* aspartic prot inase, *A. niger* neutral α-amylase, *A. niger* acid stable α-amylase, *A. niger* glucoamylase, *Rhizomucor miehei* lipase, *A. oryzae* alkaline protease, *A. oryzae* triose phosphate isomerase or *A. nidulans* acetamidase.

Expression vector

The expression vector of the invention may also comprise a suitable transcription terminator and, in eukaryotes, polyadenylation sequences operably connected to the DNA sequence encoding the α-amylase variant of the invention. Termination and polyadenylation sequences may suitably be derived from the same sources as the promoter.

The vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. Examples of such sequences are the origins of replication of plasmids pUC19, pACYC177, pUB110, pE194, pAMB1 and pIJ702.

The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the *dal* genes from *B. subtilis* or *B. licheniformis*, or one which confers antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracyclin resistance. Furthermore, the vector may comprise *Aspergillus* selection markers such as amdS, argB, niaD and sC, a marker giving rise to hygromycin resistance, or the selection may be accomplished by co-transformation, *e.g.* as described in WO 91/17243.

The procedures used to ligate the DNA construct of the invention encoding a cutinase variant, the promoter, terminator and other elements, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989).

Host Cells

The cell of the invention, either comprising a DNA construct or an expression vector of the invention as defined above, is advantageously used as a host cell in the recombinant production of a cutinase variant of the invention. The cell may be transformed with the DNA construct of the invention encoding the variant, conveniently by integrating the DNA construct (in one or more copies) in the host chromosom . This integration is generally considered to be an advantage as the DNA sequence is more likely to be stably maintained in the cell. Integration of the DNA constructs into the host chromosome may be performed according to conventional

methods, *e.g.* by homologous or heterologous recombination. Alternatively, the cell may be transformed with an expression vector as described above in connection with the different types of host cells.

The cell of the invention may be a cell of a higher organism such as a mammal or an insect, but is preferably a microbial cell, e.g. a bacterial or a fungal (including yeast) cell.

Examples of suitable bacteria are Gram positive bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus*, *Bacillus megaterium*, *Bacillus thuringiensis*, or *Streptomyces lividans* or *Streptomyces murinus*, or gramnegative bacteria such as *E.coli*. The transformation of the bacteria may, for instance, be effected by protoplast transformation or by using competent cells in a manner known *per se*.

The yeast organism may favorably be selected from a species of *Saccharomyces* or *Schizosaccharomyces*, *e.g. Saccharomyces cerevisiae*.

The host cell may also be a filamentous fungus *e.g.* a strain belonging to a species of *Aspergillus*, most preferably *Aspergillus oryzae* or *Aspergillus niger*, or a strain of *Fusarium*, such as a strain of *Fusarium oxysporium*, *Fusarium graminearum* (in the perfect state named *Gribberella zeae,* previously *Sphaeria zeae, synonym with Gibberella roseum and Gibberella roseum* f. sp. *cerealis)*, or *Fusarium sulphureum (*in the prefect state named *Gibberella puricaris,* synonym with *Fusarium trichothecioides, Fusarium bactridioides, Fusarium sambucium, Fusarium roseum,* and *Fusarium roseum var. graminearum), Fusarium cerealis (*synonym with *Fusarium crokkwellnse*), or *Fusarium venenatum*.

In a preferred embodiment of the invention the host cell is a protease deficient or protease minus strain.

This may for instance be the protease deficient strain *Aspergillus oryzae* JaL 125 having the alkaline protease gene named "alp" deleted. This strain is described in WO 97/35956 (Novo Nordisk).

Filam ntous fungi cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se. The use of *Aspergillus* as a host micro-organism

is described in EP 238 023 (Novo Nordisk A/S), the contents of which are hereby incorporated by reference.

Production of cutinase variant by cultivation of transformant

The invention relates, *inter alia*, to a method of producing a cutinase variant of the invention, which method comprises cultivating a host cell under conditions conducive to the production of the variant and recovering the variant from the cells and/or culture medium.

The medium used to cultivate the cells may be any conventional medium suitable for growing the host cell in question and obtaining expression of the cutinase variant of the invention. Suitable media are available from commercial suppliers or may be prepared according to published recipes (*e.g.* as described in catalogues of the American Type Culture Collection).

The cutinase variant secreted from the host cells may conveniently be recovered from the culture medium by well-known procedures, including separating the cells from the medium by centrifugation or filtration, and precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by the use of chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

Expression of variant in plants

The present invention also relates to a transgenic plant, plant part or plant cell which has been transformed with a DNA sequence encoding the variant of the invention so as to express and produce this enzyme in recoverable quantities. The enzyme may be recovered from the plant or plant part. Alternatively, the plant or plant part containing the recombinant enzyme may be used as such.

The transgenic plant can be dicotyledonous or monocotyledonous, for short a dicot or a monocot. Examples of monocot plants are grasses, such as meadow grass (blue grass, Poa), forage grass such as festuca, lolium, temperate grass, such as Agrostis, and cereals, e.g. wheat, oats, rye, barley, rice, sorghum and maize (corn).

Examples of dicot plants are tobacco, legumes, such as lupins, potato, sugar beet, pea, bean and soybean, and cruciferous (family Brassicaceae), such as cauliflower, oil seed rape and the closely related model organism Arabidopsis thaliana.

Examples of plant parts are stem, callus, leaves, root, fruits, seeds, and tubers. In the present context, also specific plant tissues, such as chloroplast, apoplast, mitochondria, vacuole, peroxisomes and cytoplasm are considered to be a plant part. Furthermore, any plant cell, whatever the tissue origin, is considered to be a plant part.

Also included within the scope of the invention are the progeny of such plants, plant parts and plant cells.

The transgenic plant or plant cell expressing the variant of the invention may be constructed in accordance with methods known in the art. In short the plant or plant cell is constructed by incorporating one or more expression constructs encoding the enzyme of the invention into the plant host genome and propagating the resulting modified plant or plant cell into a transgenic plant or plant cell.

Conveniently, the expression construct is a DNA construct which comprises a gene encoding the enzyme of the invention in operable association with appropriate regulatory sequences required for expression of the gene in the plant or plant part of choice. Furthermore, the expression construct may comprise a selectable marker useful for identifying host cells into which the expression construct has been integrated and DNA sequences necessary for introduction of the construct into the plant in question (the latter depends on the DNA introduction method to be used).

The choice of regulatory sequences, such as promoter and terminator sequences and optionally signal or transit sequences is determined, eg on the basis of when, where and how the enzyme is desired to be expressed. For instance, the expression of the gene encoding the enzyme of the invention may be constitutive or inducible, or may be developmental, stage or tissue specific, and the gene product may be targeted to a specific tissue or plant part such as seeds or leaves. Regulatory sequ nces are g described by Tague et al, Plant, Phys., 86, 506, 1988.

For constitutive expression the 35S-CaMV promoter may be used (Franck et al., 1980. Cell 21: 285-294). Organ-specific promoters may eg be a promoter from

storage sink tissues such as seeds, potato tubers, and fruits (Edwards & Coruzzi, 1990. Annu. Rev. Genet. 24: 275-303), or from metabolic sink tissues such as meristems (Ito et al., 1994. Plant Mol. Biol. 24: 863-878), a seed specific promoter such as the glutelin, prolamin, globulin or albumin promoter from rice (Wu et al., Plant and Cell Physiology Vol. 39, No. 8 pp. 885-889 (1998)), a *Vicia faba* promoter from the legumin B4 and the unknown seed protein gene from *Vicia faba* described by Conrad U. et al, Journal of Plant Physiology Vol. 152, No. 6 pp. 708-711 (1998), a promotter from a seed oil body protein (Chen et al., Plant and cell physiology vol. 39, No. 9 pp. 935-941 (1998), the storage protein napA promoter from Brassica napus, or any other seed specific promoter known in the art, eg as described in WO 91/14772. Furthermore, the promoter may be a leaf specific promoter such as the rbcs promoter from rice or tomato (Kyozuka et al., Plant Physiology Vol. 102, No. 3 pp. 991-1000 (1993), the chlorella virus adenine methyltransferase gene promoter (Mitra, A. and Higgins, DW, Plant Molecular Biology Vol. 26, No. 1 pp. 85-93 (1994), or the aldP gene promoter from rice (Kagaya et al., Molecular and General Genetics Vol. 248, No. 6 pp. 668-674 (1995), or a wound inducible promoter such as the potato pin2 promoter (Xu et al, Plant Molecular Biology Vol. 22, No. 4 pp. 573-588 (1993).

A promoter enhancer element may be used to achieve higher expression of the enzyme in the plant. For instance, the promoter enhancer element may be an intron which is placed between the promoter and the nucleotide sequence encoding the enzyme. For instance, Xu et al. *op cit* disclose the use of the first intron of the rice actin 1 gene to enhance expression.

The selectable marker gene and any other parts of the expression construct may be chosen from those available in the art.

The DNA construct is incorporated into the plant genome according to conventional techniques known in the art, including *Agrobacterium*-mediated transformation, virus-mediated transformation, micro injection, particle bombardment, biolistic transformation, and electroporation (Gasser et al, Science, 244, 1293; Potrykus, Bio/Techn. 8, 535, 1990; Shimamoto et al, Nature, 338, 274, 1989).

Presently, *Agrobacterium tumefaciens* mediated gene transfer is the method of choice for generating transgenic dicots (for review Hooykas & Schilperoort, 1992.

Plant Mol. Biol. 19: 15-38), however it can also be used for transforming monocots, although other transformation methods are generally preferred for these plants. Presently, the method of choice for generating transgenic monocots is particle bombardment (microscopic gold or tungsten particles coated with the transforming DNA) of embryonic calli or developing embryos (Christou, 1992. Plant J. 2: 275-281; Shimamoto, 1994. Curr. Opin. Biotechnol. 5: 158-162; Vasil et al., 1992. Bio/Technology 10: 667-674). An alternative method for transformation of monocots is based on protoplast transformation as described by Omirulleh S, et al., Plant Molecular biology Vol. 21, No. 3 pp. 415-428 (1993).

Following transformation, the transformants having incorporated the expression construct are selected and regenerated into whole plants according to methods well-known in the art.

MATERIALS AND METHODS

Plasmids

pJSO026

This is a *S. cerevisiae* expression plasmid described in WO 97/07205 and in J.S.Okkels, (1996) "A URA3-promoter deletion in a pYES vector increases the expression level of a fungal lipase in Saccharomyces cerevisiae. Recombinant DNA Biotechnology III: The Integration of Biological and Engineering Sciences, vol. 782 of the Annals of the New York Academy of Sciences).

pFuku83

This is a yeast and E. coli shuttle vector for expression of the H. insolens cutinase under the control of a TPI promoter, constructed from pJSO026.

Substrate

BETEB

T rephthalic acid bis(2-hydroxyethyl)ester dibenzoate is herein abbreviated as BETEB (benzoyl-ethylene-t rephthalic-ethelene-benzoate). It was prepared from terephthalic acid bis (2-hydroxyethyl) ester and benzoic acid.

Lipase activity (LU)

A substrate for lipase is prepared by emulsifying tributyrin (glycerin tributyrate) using gum Arabic as emulsifier. The hydrolysis of tributyrin at 30 °C at pH 7 is followed in a pH-stat titration experiment. One unit of lipase activity (1 LU) equals the amount of enzyme capable of releasing 1 µmol butyric acid/min at the standard conditions.

Differential scanning calorimetry (DSC)

Sample and reference solutions are carefully degassed immediately prior to loading of samples into the calorimeter (reference: buffer without enzyme). Sample and reference solutions (approx. 0.5 ml) are thermally pre-equillibrated for 20 minutes at 5°C. The DSC scan is performed from 5 C to 95 C at a scan rate of approx. 90 K/hr. Denaturation temperatures are determined at an accuracy of approx. +/- 1 C. A VP-DSC from MicroCal Inc. is suitable for the experiments.

Methods

PCR conditions

step 1: 94° C, 120 sec.

step 2: 94° C, 60 sec

step 3: 50° C, 60 sec

step 4: 72° C, 150 sec.

Go to step 2, 35 cycles

step 5: 72° C, 480 sec.

Step 6: 4° C, for ever

EXAMPLES

Example 1: Preparation of cutinase variants

A DNA sequence encoding *H. insolens* cutinase was obtained as described in US 5,827,719 (Novo Nordisk) and was found to have the DNA sequence shown in SEQ ID NO: 1 therein.

Variants were prepared by localized random mutagenesis and selection of positive clones by incubation at 60°C for 1 day on BETEB plates. The BETEB plates contained 200 ml/l of 500 mM glycine buffer (pH 8.5), 1.25 g/l of BETEB (dissolved in hot ethanol) and 20 g/l of agar.

Three positive variants were isolated, and their amino acid sequence was determined. They were found to have the following modifications, compared to the parent *H. insolens* cutinase:

A14P + E47K

E47K

E179Q

Example 2: Site directed mutation

A variant of the *H.* insolens cutinase having the substitutions E6Q+ E47K+ R51P was prepared as follows:

A pair of PCR primers were designed so as to introduce amino acid substitutions, making use of the existed restriction enzyme sites nearby, as follows (an asterisk indicates an introduced mutation):

Upper primer: E6Q F

cgg cag ctg gga gcc atc c*ag aac

Pvu II

Lower primer: E47K,R51P

cgc cct gga tcc aga tgt tcg* gga tgt ggg act t*aa ggc

*Bam*H I

PCR was run using these primers and pFukuNL83 as a template under the PCR condition described above.

The obtained PCR fragment was purified by Clontech Spincolumn and digested with *Pvu* II and *Bam*H I.

The resultant fragment was gel-purified and ligated to pFukuNL83 which had b en digested with the same restriction enzyme sites.

Exampl 3: Therm stability of cutinase variants

Variants

The thermostability was tested as described below for the *H. insolens* cutinase and the following variants thereof:

A14P+ E47K

E47K

E179Q

E6Q+ E47K+ R51P

A14P+ E47K+ E179Q

E6Q+ A14P+ E47K+ R51P+ E179Q

E6Q+ E10Q+ A14P+ E47K+ R51P+ E179Q

Differential Scanning Calorimetry (DSC)

Thermostability of cutinase variants was investigated by means of DSC at pH 4.5 (50 mM acetate buffer) and pH 8.5 (50mM glycyl-glycine buffer). The thermal denaturation temperature, T_d, was taken as the top of denaturation peak (major endothermic peak) in thermograms (Cp vs. T) obtained after heating of enzyme solutions at a constant programmed heating rate.

The parent cutinase was found to have T_d of 63°C at pH 8.5. Six of the above variants were found to have T_d of 70-73°C, i.e. an improvement of 7-10°.

The parent cutinase was found to have T_d of 61°C at pH 4.5. Five of the above variants were found to have T_d of 64-66°C, i.e. an improvement of 3-5°.

Hydrolysis of BETEB

The thermostability of the *H. insolens* cutinase and two of the above variants was measured by hydrolysis of BETEB at elevated temperature. For each cutinase, the following mixture was incubated for 17 hours at various temperatures in the range 55-70°C:

0.1 ml 0.5 M glycyl-glycine buffer (pH 8.5)

0.1 ml 0.5 % BETEB dissolved in ethanol

0.1 ml enzyme solution (approx. 25 LU/ml)

0.7 ml Milli Q water

The d gree of hydrolysis was measured after the incubation. The results are shown in the table below.

	Variant	Variant	Parent
	27 LU/ml	25 LU/ml	24 LU/ml
55°C	98 %	99 %	72 %
60°C	91 %	83 %	33 %
65°C	66 %	13 %	7 %

These results clearly show that the variants have improved thermostability compared to the parent cutinase.

Hydrolysis of BETEB

The thermostability of the *H. insolens* cutinase and three of the above variants was measured by hydrolysis of BETEB at 60°C for 2 hours. The hydrolysis was carried out at the above conditions, except that the temperature was fixed at 60°C and the cutinase dosage was varied. The results below are shown in the table below.

LU/ml	Variant	Variant	Variant	Parent
0	0 %	0 %	0 %	0 %
10	97 %	99 %	9 %	6 %
20	98 %	99 %	74 %	
50	98 %	94 %	93 %	15 %
100	88 %	69 %	92 %	34 %
300				41 %
600				63 %
1200				82 %

The results show a much faster hydrolysis at 60°C with the variants than with the parent cutinase.

Example 4: Hydrolysis of c3ET

The *H. insolens* cutinase and five of the above variants were tested in hydrolysis of c3ET at elevated temperature. For each cutinase, the following mixture was incubated for 2 hours at various temperatures.

0.115mg c3ET (0.1ml of 2mM c3ET dissolved in HFIP was taken in reaction vessel. Solvent was removed under vacuum, then dried up at 70°C over night)

0.1ml 0.5M glycyl-glycine buffer (pH8.5)

0.1ml enzyme solution (approx. 600LU/ml)

0.8ml Milli Q water

After the incubation, 2ml of 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was added to each reaction mixture, then hydrolysis ratio was measured by HPLC. The results shown in Fig 3 clearly indicate that the variants have improved thermostability compared to the parent cutinase.

Example 5: Hydrolysis of c3ET on yarn

The thermostability of the *H. insolens* cutinase five of the above variants was tested using polyester yarn containing c3ET as by product. The following substrate mixture was preincubated at 60 or 65°C:

0.1g polyester yarn

0.2ml 0.5M glycyl-glycine buffer (pH8.5)

1.7ml Milli Q water

After preincubation, 0.1ml enzyme solution (approx. 1000 LU/ml) was added to each reaction vessel and incubated for 17 hours. Then 2ml HFIP was added and left for 30 minutes to extract and hydrolyze c3ET sitting on the surface of the polyester yarn; then the hydrolysis ratio was measured. The results are shown in Fig. 4.

It is seen that the variants are more effective than the parent cutinase for hydrolyzing c3ET on polyester yarn. One variant gives higher hydrolysis ratio at 65°C than at 60°C.

Example 6: Treatment of yarn with cutinase variant

Time courses of c3ET hydrolysis on polyester yarn at different temperature or dosage were examined. Time course at different temperatures is shown in Fig 5. It is seen that the optimum temperature is 65°C. At 70°C there is still about half of the activity left. Time course with increased enzyme dosage is shown in Fig 6. The curves at dosage 275 and 550 LU/ml are seen to be the same, indicating that the hydrolysis ratio reached to plateau between dosage of 100 to 275 LU/ml. Presumably 200LU/ml is enough.

Example 7: Dyeing polyester with reactive dye

The following polyester fabrics were treated:

woven fabric; ca. 2 x 2 cm, 34mg

knitted fabric; ca. 1.5 x 1.5 cm, 50mg

Each fabric was soaked in 0.9 ml, 50 mM GlyGly (glycyl-glycine) buffer (pH 8.5) and 0.1 ml solution of a variant of the *H. insolens* cutinase (1100 LU/ml), and incubated at 65 or 70°C. After one day, another 0.1 ml enzyme solution was added, incubation was continued for two more days, the fabrics were then taken out and rinsed in water. A comparative experiment was made with the parent cutinase, and a blank was treated in the same manner without enzyme.

The fabrics were stirred in a mixture of 9 g 120 g Na_2SO_4 and 60 g Na_2CO_3 in 3 liter deionized water at 60 °C for 30 min, and then rinsed with running warm water. The reactive dye was Celmazol Brilliant Blue B (product of Mitsui Chemical Co., Japan), which has the structure Chromophore-NHPh-$SO_2CH_2CH_2OSO_3Na$.

In all four experiments, (woven and knitted, 65 and 70°C), the fabrics were uniformly dyed.

Example 8: Solubilization of polyester fragments from knitted textile

A 1x1 cm sample of knitted polyester textile (PET, polymer of ethyleneglycol and terephthalic acid) was incubated for 1 hour in 1 ml of buffer at pH 10, 60°C with 0.01 mg of a variant of *H. insolens* cutinase. The reaction mixture was separated, and the rel ase of terephthalic acid was found by measuring OD at 250 nm (ex-

pressed as OD_{250}/mg PET). comparative experiments were made without nzyme or with the parent cutinase. Results:

	Enzyme	OD_{250}
Invention	Cutinase variant	4.5
Reference	Parent cutinase	0.3
	None	0.1

The results show that the variant is effective in solubilizing polyester.

In another experiment, the cutinase variant was tested for 2 hours at 65°C with and without the addition of a non-ionic surfactant (alcohol ethoxylate, product name Softanol 50), using various amounts of the variant from 0.5 to 200 LU/ml. The results showed more solubilization in the presence of non-ionic surfactant.

Example 9: Hydrolysis of polycaprolactone and polyester film

About 0.1 g of polycaprolactone or polyester film were put in tubes. They were soaked in 5ml of 50mM GlyGly buffer (pH 8.5) with or without a variant of *H. insolens* cutinase (450 LU). They were incubated at 70°C for 5 hours. After the reaction we observed a thin layer of hydrolysate on the surface of the tubes with enzyme, both with polycaprolactone and with polyester film. On the other hand no change was observed in controls without enzyme. In the case of polycaprolactone there was 10% of weight loss. We see no weight change of polyester.

Example 10: cPET hydrolysis

The performance of a cutinase variant was compared with the parent enzyme (*H. insolens* cutinase). The trials were done as follows:

An oligomer-stained swatch of (black) PET-fabric (app. 4cm x 13cm) is subjected to the enzyme-treatment at relatively low agitation in a so-called mini-tergitometer apparatus. The PET-fabric is mounted onto a cylindrical, perforated holder (radius ca.2 cm, height ca 6 cm) , that rotates around its axis, and with the oligomer stained side of the PET fabric facing the exterior of the cylinder.

The fabric is immersed in a 150ml glass-beaker containing 100ml of the treatment solution at a given temperature (here 65°C). After a given treatment time

(here 90minutes) the PET swatch is removed from the bath and rinsed in deionized water and air dried.

After conditioning the swatches are visually ranked (with respect to oligomer stain removal) on the side having the oligomer-staining. The rating being as follows:

-2: Sample significantly worse than blank (no enzyme)

-1: Sample slightly worse than blank (no enzyme)

0: Sample can not be distinguished from blank

1: Sample slightly improved vs blank

2: Sample significantly improved over blank

Also, the swatches are read spectrofotometrically (apparatus: Hunterlab Reflectometer) to quantify the color strength (K/S-value at 600nm).

The table below summarizes the test-conditions for a trial comparing the performance the enzymes under similar conditions:

Temperature:	65°C
Buffer/pH:	50 mM glycine buffer, pH 10.3
Treatment time (min)	90
Dosage of Enzyme (LU/g)	30000

Results from the trial are summarized below

Enzyme	Visual rating (avg.)	K/S Difference @ 600 nm
None	0 (defined)	2.33
Parent cutinase	0	2.38
Cutinase variant	1.5-2.0	2.89

From this set of experiments it thus appears that the parent enzyme provides no or only very limited effect at the given test conditions (probably because the temperature is too high for the enzyme to retain activity), while the cutinase variant provides a substantial removal of the oligomer staining from the PET-fabric.

Exampl 11: cPET hydrolysis

The pH and temperature profile of a variant of *H. insolens* cutinase was tested in a model disperse dyeing experiment. The trials were performed as follows:

An oligomer-stained swatch of (black) PET-fabric is subjected to the conditions of a typical disperse dyeing sequence in a Werner Mathis Labomat. In overview of the process, the swatch is added to a buffer solution, heated to 130°C, cooled down to the treatment temperature. Enzyme or buffer is added and then held at the desired temperature for 30 minutes. The solution is cooled down to room temperature and turbidity in the wash liquor is measured. The reduction in turbidity is a direct measure of the cutinase activity, corresponding to hydrolyzed cPET oligomers.

Detailed description of the experiment:

A black PET (app. 4cm x 13cm) swatch is added 140 ml 100 mM Britton-Robinson buffer containing 0.2 g/l Lutensol AT11 (BASF) and loaded in the Labomat (32 rotation per minute).

The Labomat is heated to 130°C at a gradient of 9°C/minute, and held for 10 minutes.

The beakers are cooled to run temperature (according to table below) at a gradient of 9°C/minute, and held for 1 minute.

10 mL enzyme solution (100 LU/ml of the variant) or buffer solution (0 LU/ml) at appropriate pH is injected to the beakers.

The Labomat is re-heated to temperature at a gradient of 2°C/minute, and held for 30 minutes.

The swatches are removed, and the wash liquor is cooled down to room temperature.

Turbidity of the wash liquors are measured.

Evaluation: Turbidity is measured on Hach 18900 Ratio Turbidimeter (standardized with 1.8, 18, and 180 NTU Turbidity Standards). Enzyme performance is calculated relative to a blank as the difference between turbidity of blank liquor (no enzyme) and turbidity of enzyme treated liquor.

The relative performance (reduction in turbidity) of the cutinase variant is calculated, and the results are shown in the following table. When a negative num-

ber is obtained, then the result is given as "negative". A negative number is assumed to be an artifact, caused by the variation of the set up.

Temperature	pH 7	pH 8	pH 9	pH 10
60°C	39	57	37	14
65°C	39	16	60	30
70°C	25	12	54	33
75°C	22	50	114	58
85°C	negative	negative	15	negative

The results show that the cutinase variant is active over a broad pH and temperature range, with optimum oligomer removal in the current set up around pH 9 and 75°C. Inactivation seems to occur at or above 85°C.

Example 12: cPET hydrolysis

The effect of treatment time was investigated for a variant of *H. insolens* cutinase in a model disperse dyeing experiment. The trials were performed as follows:

An oligomer-stained swatch of (black) PET-fabric is subjected to the conditions of a typical disperse dyeing sequence in a Werner Mathis Labomat. In overview of the process, the swatch is added to a buffer solution, heated to 130°C, cooled down to the treatment temperature. Enzyme or buffer (100 mM Britton-Robinson pH 9) is added, and then held at 75°C for 0-40 minutes. The solution is cooled down to room temperature and turbidity in the wash liquor is measured. The reduction in turbidity is a direct measure of the cutinase activity, corresponding to hydrolyzed cPET oligomers.

<u>Detailed description of the experiment:</u>

A black PET (app. 4cm x 13cm) swatch is added to 140 ml 100 mM Britton-Robinson buffer containing 0.2 g/l Lutensol AT11 (BASF) and loaded in the Labomat (32 rotation per minute).

The Labomat is heated to 130°C at a gradient of 9°C/minute, and the temperature is held for 10 minutes.

The b akers are cooled to 75°C at a gradient of 9°C/minute, and held for 1 minute.

10 mL enzyme solution (100 LU/ml of variant) or 100 mM Britton-Robinson buffer pH 9.0 (0 LU/ml) is injected into the beakers.

The Labomat is re-heated to 75°C at a gradient of 2°C/minute, and held for the appropriate number of minutes (0-40 minutes, see table below).

The swatches are removed, and the wash liquor is cooled down to room temperature.

Turbidity of the wash liquors are measured.

Evaluation: Turbidity is measured on Hach 18900 Ratio Turbidimeter (standardized with 1.8, 18, and 180 NTU Turbidity Standards). Enzyme performance is calculated relative to a blank at time equal to zero: Turbidity of blank liquor at time zero (no enzyme) subtracted turbidity of enzyme treated liquor (at a given time).

The relative performance (reduction in turbidity) of the cutinase variant was calculated, and the results are shown in the following table.

Time (minutes)	Relative performance (Reduction in turbidity)
0	0
5	42
10	48
15	62
20	69
25	85
30	72
40	78

The results show that the effect of the enzyme is increased over time. At the current enzyme dose and oligomer concentration, it seems to level off above approx. 20 minutes.

Example 13: Fiber modification

The effect on wetting characteristics of a disperse dyed polyester fabric was investigated by treating the fabric with a variant of *H. insolens* cutinase prior to dy -

ing. Th experiment therefore consisted of two phases, the actual fiber modification and the disperse dyeing pro- cedure.

Phase 1 - Fiber Modification:

Equipment:	Atlas Launder-O-meter LP2
Fabric:	knit 100 % scoured polyester from Testfabrics
pH:	50 mM potassium phosphate buffer, pH 7
Abrasives:	5 big steel balls
Beaker Vol.:	120 mL
Treatment:	2 hours 65°C then ramped up to 90°C and held for 1 hour
Swatch Prep:	Cut 3* 1.5 g swatch of fabric, 3 per beaker = 4.5 g

Rinse:

Rinse in deionized water.

Phase 2 - Dyeing - disperse dye:

Dye Solution:

Add together with deionized water to make liquor ratio 1:20-

0.4 % Dianix Red (DyStar) SE-CB (owf)

pH to 4.5 - 5

Dyeing Procedure:

1. One swatch per treatment from the fiber modification is used for the dyeing (1.5 g/swatch is used for the liquor ratio calculation).

2. Make dyebath according to the recipe above. Add the cold dye solution to the Labomat beakers and heat to 55°C at a gradient of 3.5°C/minute. Run for 5 minutes once temperature has been reached.

3. Add the fabric to the beaker.

4. Raise temperature to 130°C at a gradient of 1.5°C/minute. Dye for 30 minutes.

5. Cool to 70°C at a gradient of 5°C/minute. Drop bath, but collect, and rinse fabric hot (60°C) for 10 minutes. Follow th hot rinse with a room t mperature overflow rins until all bleeding had stopped.

6. L t air dry overnight.

Tests/Analysis:

AATCC Test Method 61 - Colorfastness to washing

Percent Dyebath Exhaustion - Spectrophotometer

K/S and L* - Reflectometer

AATCC TM-79 Drop Test

Results:

The results from the fiber modification are shown in the following table.

Variant dosage	Staining (AATCC TM-61)	Color Change (K/S @ 530 before and after TM-61)	Drop Test (AATCC TM-79)
Blank	4.5	5	53 sec.
50 LU/mL	4.5	5	18 sec.
100 LU/mL	4.5	5	15 sec.

The results show that the treatment of polyester with the variant increases the wetting substantially. No adverse effects are noticed on the dyeability with the disperse dye in the current set-up.

Example 14: Malodor reduction in textiles soiled with human sweat/sebum by use of a cutinase variant in laundry

The performance of cutinase, with respect to malodor reduction, can be tested in a one-cycle washing trial carried out in a Terg-O-tometer.

Experimental conditions:

Washing liquor: 1000 ml per beaker

Swatches: 100 % polyester (interlock knitted, previously cleaned by Soxhlet extraction). 24 swatches (3.3 × 3.5 cm) per beaker.

Soil: Human male axillary sweat and sebum applied by wiping the armpits aft r exercise.

Detergent: 5 g/L of a standard color d tergent. No pH adjustm nt.

Wat r hardness: 3.2 mM Ca^{2+}/Mg^{2+} (in a ratio of 5:1)

Wash T mperature: 30°C

Wash tim : 30 min

Rins : 15 minutes in running tap water

Evaluation:

After wash the wet swatches are placed in closed, tinted 200 ml glasses. A trained sensory panel (9-11 judges) evaluates the odor by sniffing the headspace over the wet samples and evaluates the total odor intensity. The odor intensity is noted by placing a mark on an unstructured line scale measuring 15 cm, with word anchors at each end ('nothing' at the beginning of the scale and 'very strong' at the end). All evaluations are performed twice. The swatches are evaluated on day 1, 2 and 3 after wash (swatches are kept in the glasses at all times).

CLAIMS

1. A variant of a parent fungal cutinase, which variant:

a) comprises substitution of one or more amino acid residues at a position which is located:

i) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

ii) within 20 positions from the N-terminal amino acid, and

b) is more thermostable than the parent cutinase.

2. The variant of the preceding claim which comprises substitution of one or more amino acid residues at a position which is located:

i) within 12 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

ii) within 15 positions from the N-terminal amino acid.

3. A variant of a parent fungal cutinase comprising substitution of one or more amino acid residues which is located:

a) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

b) within 20 positions from the N-terminal amino acid,

with the proviso that it is not a variant of the cutinase of *Fusarium solani pisi* having one of the substitutions R17, T18, T19V, D21N, I24E, Y38F, R40, G41A, S42, T43, E44, T45, G46, N47R, G49, T50, L51, P53, S54, A56C, S57, N58R, S61, A62E, K65A, D66S, G67D, W69Y, I70C, G74, G75, R78, Y119, G192, P193, D194R, A195, R196, G197V, or A199C (*Fusarium solani pisi* cutinase numbering).

4. A variant of a parent fungal cutinase comprising substitution of one or more amino acid residues which:

a) has a solvent accessible surface, and

b) is located:

i) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

ii) within 20 positions from the N-terminal amino acid,

with the proviso that it is not a variant of the cutinase of *Fusarium solani pisi* having one of the substitutions T18, Y38F, R40, G41A, S42, T43, E44, T45, N47R, G49, T50, L51, P53, S54, A56C, A62E or G192 (*Fusarium solani pisi* cutinase numbering).

5. A variant of a parent fungal cutinase comprising substitution of one or more amino acid residues which is located:

a) less than 12 Å from the location of the N-terminal amino group (as calculated from amino acid residues in a crystal structure), and/or

b) within 15 positions from the N-terminal amino acid,

with the proviso that the variant is not the cutinase of *Fusarium solani pisi* having one of the substitutions R17, T18, T19V, D21N, Y38F, R40, T45, G46, N47R, G49, T50, L51, P53, S54, A56C, S57, N58R, K65A or I70C (*Fusarium solani pisi* cutinase numbering).

6. The variant of any preceding claim wherein the parent cutinase is native to a filamentous fungus, preferably a strain of *Humicola* or *Fusarium*, preferably *H. insolens* or *F. solani pisi*, most preferably *H. insolens* strain DSM 1800.

7. The variant of any preceding claim wherein the parent cutinase has an amino acid sequence which can be aligned with the cutinase of *H. insolens* strain DSM 1800.

8. Th variant of any preceding claim wherein the parent cutinase has an amino acid sequence which is at least 50 % homologous to the cutinase of *H. insolens* strain DSM 1800, preferably at least 70 % homologous, more preferably at least 80 % homologous.

9. A variant of a parent fungal cutinase from *Humicola insolens* which comprises substitution of one or more amino acid residues located:

a) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

b) within 20 positions from the N-terminal amino acid.

10. The variant of the preceding claim which comprises substitution of one or more amino acid residues located:

a) less than 12 Å from the location of the N-terminal amino group (as calculated from amino acid residues in a crystal structure), and/or

b) within 15 positions from the N-terminal amino acid

11. The variant of any preceding claim which comprises substitution of one or more amino acids having a solvent accessible surface.

12. The variant of any preceding claim wherein one or more substitutions is substitution of a negatively charged amino acid with a neutral or positively charged amino acid or substitution of a neutral amino acid with a positively charged amino acid.

13. The variant of the preceding claim wherein one or more substitutions is at a position corresponding to position E6, E10, E30, E47, D63, E82 and/or E179 in the cutinase of *Humicola insolens* strain DSM 1800, preferably a substitution with R/K/Y/H/Q/N, more preferably a substitution corresponding to E6N/Q, E10N/Q, E47K/R and/or E179N/Q (*H. insolens* cutinase numbering).

14. The variant of any preceding claim wherein one or more substitutions is substitution with a Pro residue, preferably at a position corresponding to position A14 and/or R51.

15. The variant of any preceding claim which has one, two, three, four, five or six of said substitutions.

16. The variant of any preceding claim which has substitutions corresponding to one of the following in the cutinase of *Humicola insolens* strain DSM 1800:

a) R51P

b) E6N/Q + L138I

c) A14P + E47K

d) E47K

e) E179N/Q

f) E6N/Q + E47K + R51P

g) A14P + E47K + E179N/Q

h) E47K + E179N/Q

i) E47K + D63N

j) E6N/Q + A14P + E47K + R51P + E179N/Q

k) E6N/Q + E10N/Q + A14P + E47K + R51P + E179N/Q, or

l) Q1P + L2V + S11C + N15T + F24Y + L46I + E47K

17. The variant of any preceding claim which has hydrolytic activity towards terephthalic acid esters, particularly towards cyclic tri(ethylene terephthalate) and/or Terephthalic acid bis(2-hydroxyethyl)ester dibenzoate (BETEB).

18. The variant of any preceding claim which has a denaturation temperature which is at least 5° higher than the parent cutinase, preferably measured at pH 8.5

19. A DNA sequence encoding the variant of any preceding claim.

20. A vector comprising the DNA sequence of the preceding claim.

21. A transformed host cell harboring the DNA sequence of claim 19 or the vector of claim 20.

22. A method of producing the variant of any of claims 1-18 comprising

a) cultivating the cell of claim 21 so as to express and preferably secrete the variant, and

b) recovering the variant.

23. A method of constructing a cutinase variant, which method comprises:

a) selecting a parent fungal cutinase,

b) identifying one or more amino acid residues in the parent cutinase at positions which are:

i) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

ii) within 20 positions from the N-terminal amino acid, and

c) making alterations each of which is an insertion, a deletion or a substitution of the amino acid residue,

d) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

e) preparing the variant resulting from steps b-d,

f) testing the thermostability of the variant,

g) optionally repeating steps b-f, and

h) selecting a variant having higher thermostability than the parent cutinase.

24. A method of producing a cutinase variant, which method comprises:

a) selecting a parent fungal cutinase,

b) identifying one or more amino acid residues in the parent cutinase at positions which are:

i) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

ii) within 20 positions from the N-terminal amino acid, and

c) making alterations each of which is an insertion, a deletion or a substitution of the amino acid residue,

d) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

e) preparing the variant resulting from steps b-d,

f) testing the thermostability of the variant,

g) optionally repeating steps b-f,

h) selecting a variant having higher thermostability than the parent cutinase, and

i) producing the variant to obtain the cutinase variant.

25. A process for enzymatic hydrolysis of a cyclic oligomer of poly(ethylene terephthalate), which process comprises treating the cyclic oligomer with a variant of a parent fungal cutinase, which variant comprises substitution of one or more amino acid residues at a position which is located:

i) within 17 Å from the location of the N-terminal amino acid (as calculated from amino acid residues in a crystal structure), and/or

ii) within 20 positions from the N-terminal amino acid.

26. The process of the preceding claim, in which the cyclic oligomer is cyclic tri(ethylene terephthalate).

27. The process of claim 25 or 26 wherein the treatment is done at 60-80°C, preferably at 65-75°C.

28. The process of any of claims 25-27 wherein the cyclic oligomer is present in and on the fibers of a polyester containing fabric or yarn.

29. The process of any of claims 25-28 which further comprises subsequently rinsing the fabric or yarn, preferably rinsing with an aqueous solution having a pH in the rang of from about pH 7 to about pH 11.

30. A process for dyeing polyester fabric or yarn, comprising:

a) treating the fabric or yarn with a cutinase having a thermal denaturation temperature of 65°C or higher at pH 8.5; and

b) dyeing the treated fabric with a reactive dye or a disperse dye.

31. The process of the preceding claim wherein the cutinase is the variant of any of claims 1-18.

32. A detergent composition comprising a surfactant and the variant of any of claims 1-18.

33. A method for detecting cutinase activity in a sample, comprising incubating the sample with terephthalic acid bis(2-hydroxyethyl)ester dibenzoate and detecting hydrolysis of said ester.

34. A process for improving the functional finish of a PET-containing yarn or fabric comprising

a) treating the yarn or fabric with the variant of any of claims 1-18, and

b) subsequently the yarn or fabric with a finishing agent selected from the group consisting of softeners, anti-crease resins, anti-static agents, anti-soiling agents.

Fig. 1

3D structure of cutinase from *Humicola insolens*

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ATOM      1  N   GLY A   3      24.424  -7.935  18.390  1.00 46.73
ATOM      2  CA  GLY A   3      23.848  -8.994  17.546  1.00 42.29
ATOM      3  C   GLY A   3      24.396 -10.112  16.727  1.00 37.35
ATOM      4  O   GLY A   3      25.347 -10.913  16.728  1.00 35.38
ATOM      5  N   ALA A   4      23.664 -10.625  15.797  1.00 34.53
ATOM      6  CA  ALA A   4      23.051 -10.874  14.555  1.00 30.95
ATOM      7  C   ALA A   4      21.574 -11.246  14.920  1.00 28.33
ATOM      8  O   ALA A   4      20.677 -10.499  14.446  1.00 22.94
ATOM      9  CB  ALA A   4      23.574 -11.780  13.556  1.00 26.92
ATOM     10  N   ILE A   5      21.583 -12.058  16.043  1.00 26.48
ATOM     11  CA  ILE A   5      20.281 -12.289  16.637  1.00 25.65
ATOM     12  C   ILE A   5      20.316 -12.151  18.118  1.00 22.40
ATOM     13  O   ILE A   5      21.060 -12.888  18.717  1.00 24.74
ATOM     14  CB  ILE A   5      19.724 -13.683  16.524  1.00 26.04
ATOM     15  CG1 ILE A   5      19.852 -13.927  15.050  1.00 29.85
ATOM     16  CG2 ILE A   5      18.374 -13.558  17.159  1.00 20.48
ATOM     17  CD1 ILE A   5      19.066 -15.133  14.709  1.00 27.96
ATOM     18  N   GLU A   6      19.461 -11.377  18.668  1.00 20.52
ATOM     19  CA  GLU A   6      19.207 -11.015  20.040  1.00 17.94
ATOM     20  C   GLU A   6      17.711 -11.027  20.432  1.00 17.76
ATOM     21  O   GLU A   6      16.931 -10.165  19.990  1.00 17.60
ATOM     22  CB  GLU A   6      19.809  -9.614  20.199  1.00 14.22
ATOM     23  CG  GLU A   6      21.232  -9.374  20.385  1.00 16.71
ATOM     24  CD  GLU A   6      22.148 -10.387  21.030  1.00 34.47
ATOM     25  OE1 GLU A   6      21.634 -11.347  21.693  1.00 49.57
ATOM     26  OE2 GLU A   6      23.410 -10.310  20.975  1.00 37.43
ATOM     27  N   ASN A   7      17.375 -11.895  21.333  1.00 21.67
ATOM     28  CA  ASN A   7      16.070 -11.854  21.846  1.00 24.04
ATOM     29  C   ASN A   7      15.927 -11.488  23.238  1.00 22.08
ATOM     30  O   ASN A   7      15.098 -12.179  23.820  1.00 24.00
ATOM     31  CB  ASN A   7      15.468 -13.307  21.820  1.00 25.06
ATOM     32  CG  ASN A   7      15.039 -13.160  20.341  1.00 38.52
ATOM     33  OD1 ASN A   7      15.519 -14.147  19.759  1.00 48.45
ATOM     34  ND2 ASN A   7      14.318 -12.081  19.968  1.00 36.89
ATOM     35  N   GLY A   8      16.671 -10.813  23.926  1.00 23.56
ATOM     36  CA  GLY A   8      16.654 -10.628  25.363  1.00 23.69
ATOM     37  C   GLY A   8      15.366 -10.247  25.984  1.00 22.72
ATOM     38  O   GLY A   8      14.967 -10.939  26.867  1.00 32.25
ATOM     39  N   LEU A   9      14.785  -9.144  25.755  1.00 23.61
ATOM     40  CA  LEU A   9      13.470  -8.753  26.033  1.00 23.73
ATOM     41  C   LEU A   9      12.559  -9.961  25.782  1.00 25.93
ATOM     42  O   LEU A   9      11.494 -10.054  26.480  1.00 30.47
ATOM     43  CB  LEU A   9      12.971  -7.621  25.105  1.00  5.84
ATOM     44  CG  LEU A   9      11.556  -7.227  25.470  1.00 23.25
ATOM     45  CD1 LEU A   9      11.422  -6.765  26.968  1.00 20.21
ATOM     46  CD2 LEU A   9      11.009  -6.071  24.714  1.00 17.64
ATOM     47  N   GLU A  10      12.775 -10.786  24.773  1.00 29.56
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ATOM     48  CA  GLU A  10      11.635 -11.681  24.484  1.00 33.93
ATOM     49  C   GLU A  10      11.640 -12.872  25.412  1.00 32.18
ATOM     50  O   GLU A  10      10.600 -13.159  25.996  1.00 36.67
ATOM     51  CB  GLU A  10      11.513 -11.996  23.012  1.00 40.97
ATOM     52  CG  GLU A  10      10.054 -12.303  22.745  1.00 51.96
ATOM     53  CD  GLU A  10       9.570 -11.711  21.437  1.00 54.08
ATOM     54  OE1 GLU A  10      10.488 -11.440  20.635  1.00 48.22
ATOM     55  OE2 GLU A  10       8.323 -11.643  21.471  1.00 52.39
ATOM     56  N   SER A  11      12.822 -13.334  25.688  1.00 29.58
ATOM     57  CA  SER A  11      12.993 -14.455  26.645  1.00 35.25
ATOM     58  C   SER A  11      13.403 -14.012  28.047  1.00 39.86
ATOM     59  O   SER A  11      13.688 -14.790  28.919  1.00 43.72
ATOM     60  CB  SER A  11      14.053 -15.364  25.983  1.00 33.73
ATOM     61  OG  SER A  11      15.275 -14.620  25.928  1.00 46.98
ATOM     62  N   GLY A  12      13.467 -12.802  28.456  1.00 41.40
ATOM     63  CA  GLY A  12      13.841 -12.332  29.752  1.00 45.34
ATOM     64  C   GLY A  12      12.673 -12.562  30.694  1.00 47.62
ATOM     65  O   GLY A  12      11.485 -12.335  30.335  1.00 50.76
ATOM     66  N   SER A  13      12.969 -12.900  31.936  1.00 48.09
ATOM     67  CA  SER A  13      11.974 -13.158  32.995  1.00 45.26
ATOM     68  C   SER A  13      11.509 -11.933  33.772  1.00 39.53
ATOM     69  O   SER A  13      12.563 -11.204  33.992  1.00 36.30
ATOM     70  CB  SER A  13      12.708 -14.006  34.101  1.00 51.20
ATOM     71  OG  SER A  13      12.006 -13.947  35.338  1.00 57.14
ATOM     72  N   ALA A  14      10.256 -11.785  34.214  1.00 35.22
ATOM     73  CA  ALA A  14      10.068 -10.530  34.964  1.00 34.78
ATOM     74  C   ALA A  14      10.574 -10.620  36.417  1.00 37.51
ATOM     75  O   ALA A  14      10.809  -9.584  37.113  1.00 38.41
ATOM     76  CB  ALA A  14       8.714  -9.915  34.903  1.00 32.71
ATOM     77  N   ASN A  15      11.039 -11.834  36.737  1.00 38.85
ATOM     78  CA  ASN A  15      11.715 -12.086  37.963  1.00 43.49
ATOM     79  C   ASN A  15      13.073 -11.411  37.953  1.00 46.45
ATOM     80  O   ASN A  15      13.453 -11.022  39.022  1.00 52.50
ATOM     81  CB  ASN A  15      12.088 -13.533  38.207  1.00 53.08
ATOM     82  CG  ASN A  15      10.772 -14.226  38.553  1.00 71.86
ATOM     83  OD1 ASN A  15       9.837 -13.535  38.998  1.00 71.73
ATOM     84  ND2 ASN A  15      10.866 -15.523  38.267  1.00 77.71
ATOM     85  N   ALA A  16      13.712 -11.305  36.812  1.00 46.73
ATOM     86  CA  ALA A  16      14.915 -10.470  36.743  1.00 41.22
ATOM     87  C   ALA A  16      15.031  -9.286  35.798  1.00 36.70
ATOM     88  O   ALA A  16      16.027  -9.254  35.075  1.00 37.67
ATOM     89  CB  ALA A  16      15.903 -11.545  36.301  1.00 41.80
ATOM     90  N   CYS A  17      14.300  -8.227  35.843  1.00 30.62
ATOM     91  CA  CYS A  17      14.614  -7.093  34.997  1.00 31.78
ATOM     92  C   CYS A  17      16.024  -6.579  35.149  1.00 32.94
ATOM     93  O   CYS A  17      16.744  -6.850  36.113  1.00 39.10
ATOM     94  CB  CYS A  17      13.679  -5.881  35.138  1.00 28.00
ATOM     95  SG  CYS A  17      12.048  -6.583  34.858  1.00 24.72
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ATOM	96	N	PRO	A	18	16.529	-5.910	34.092	1.00	30.49
ATOM	97	CA	PRO	A	18	17.994	-5.626	33.971	1.00	22.04
ATOM	98	C	PRO	A	18	18.178	-4.138	34.241	1.00	20.15
ATOM	99	O	PRO	A	18	17.085	-3.459	34.370	1.00	17.83
ATOM	100	CB	PRO	A	18	18.353	-6.003	32.559	1.00	19.20
ATOM	101	CG	PRO	A	18	17.044	-6.595	32.101	1.00	20.16
ATOM	102	CD	PRO	A	18	15.903	-5.936	32.792	1.00	24.35
ATOM	103	N	ASP	A	19	19.428	-3.652	34.011	1.00	14.85
ATOM	104	CA	ASP	A	19	19.451	-2.168	34.226	1.00	16.59
ATOM	105	C	ASP	A	19	18.739	-1.367	33.156	1.00	20.42
ATOM	106	O	ASP	A	19	18.311	-0.242	33.430	1.00	23.84
ATOM	107	CB	ASP	A	19	20.896	-1.818	34.485	1.00	27.25
ATOM	108	CG	ASP	A	19	21.433	-2.389	35.793	1.00	42.30
ATOM	109	OD1	ASP	A	19	21.162	-3.549	36.297	1.00	53.52
ATOM	110	OD2	ASP	A	19	22.251	-1.719	36.543	1.00	54.02
ATOM	111	N	ALA	A	20	18.646	-1.780	31.895	1.00	20.18
ATOM	112	CA	ALA	A	20	18.066	-1.036	30.809	1.00	17.43
ATOM	113	C	ALA	A	20	17.713	-2.087	29.703	1.00	16.06
ATOM	114	O	ALA	A	20	18.334	-3.172	29.860	1.00	9.45
ATOM	115	CB	ALA	A	20	18.975	-0.048	30.100	1.00	12.07
ATOM	116	N	ILE	A	21	16.814	-1.602	28.829	1.00	8.47
ATOM	117	CA	ILE	A	21	16.657	-2.583	27.753	1.00	9.23
ATOM	118	C	ILE	A	21	16.952	-1.745	26.486	1.00	14.77
ATOM	119	O	ILE	A	21	16.681	-0.473	26.403	1.00	12.01
ATOM	120	CB	ILE	A	21	15.208	-2.984	27.837	1.00	16.28
ATOM	121	CG1	ILE	A	21	14.851	-3.898	28.956	1.00	15.55
ATOM	122	CG2	ILE	A	21	14.689	-3.671	26.514	1.00	13.71
ATOM	123	CD1	ILE	A	21	13.401	-3.879	29.372	1.00	6.12
ATOM	124	N	LEU	A	22	17.432	-2.451	25.391	1.00	12.24
ATOM	125	CA	LEU	A	22	17.665	-1.774	24.087	1.00	11.27
ATOM	126	C	LEU	A	22	16.849	-2.517	23.038	1.00	14.60
ATOM	127	O	LEU	A	22	16.908	-3.781	22.850	1.00	9.78
ATOM	128	CB	LEU	A	22	19.087	-1.865	23.693	1.00	10.96
ATOM	129	CG	LEU	A	22	19.493	-1.543	22.257	1.00	10.32
ATOM	130	CD1	LEU	A	22	19.311	-0.081	21.900	1.00	4.72
ATOM	131	CD2	LEU	A	22	20.990	-1.842	22.156	1.00	7.42
ATOM	132	N	ILE	A	23	16.038	-1.815	22.242	1.00	15.13
ATOM	133	CA	ILE	A	23	15.298	-2.459	21.115	1.00	18.06
ATOM	134	C	ILE	A	23	15.916	-1.771	19.901	1.00	17.42
ATOM	135	O	ILE	A	23	16.117	-0.519	19.795	1.00	19.31
ATOM	136	CB	ILE	A	23	13.820	-2.194	21.392	1.00	18.16
ATOM	137	CG1	ILE	A	23	13.208	-3.076	22.447	1.00	14.23
ATOM	138	CG2	ILE	A	23	12.787	-2.167	20.247	1.00	13.19
ATOM	139	CD1	ILE	A	23	12.142	-2.065	22.976	1.00	20.41
ATOM	140	N	PHE	A	24	16.218	-2.548	18.940	1.00	14.59
ATOM	141	CA	PHE	A	24	16.859	-2.159	17.671	1.00	11.72
ATOM	142	C	PHE	A	24	16.347	-2.719	16.353	1.00	7.25
ATOM	143	O	PHE	A	24	16.095	-3.998	16.161	1.00	3.47

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ATOM    144  CB  PHE A  24      18.195  -2.855  17.658  1.00 12.61
ATOM    145  CG  PHE A  24      19.015  -2.150  16.716  1.00 10.72
ATOM    146  CD1 PHE A  24      19.457  -0.844  16.913  1.00 13.08
ATOM    147  CD2 PHE A  24      19.325  -2.852  15.558  1.00  6.61
ATOM    148  CE1 PHE A  24      20.232  -0.187  15.983  1.00  4.86
ATOM    149  CE2 PHE A  24      20.061  -2.218  14.545  1.00  7.61
ATOM    150  CZ  PHE A  24      20.550  -0.823  14.804  1.00  8.78
ATOM    151  N   ALA A  25      16.037  -1.700  15.449  1.00  6.32
ATOM    152  CA  ALA A  25      15.662  -2.158  14.068  1.00  7.18
ATOM    153  C   ALA A  25      16.851  -1.976  13.055  1.00  8.59
ATOM    154  O   ALA A  25      17.518  -1.000  13.133  1.00  5.95
ATOM    155  CB  ALA A  25      14.488  -1.402  13.562  1.00  8.27
ATOM    156  N   ARG A  26      17.174  -3.032  12.325  1.00  8.84
ATOM    157  CA  ARG A  26      18.134  -3.278  11.277  1.00  4.04
ATOM    158  C   ARG A  26      17.691  -2.694   9.894  1.00  7.67
ATOM    159  O   ARG A  26      16.527  -2.361   9.525  1.00  9.36
ATOM    160  CB  ARG A  26      18.581  -4.659  10.756  1.00  6.06
ATOM    161  CG  ARG A  26      17.705  -5.741  10.439  1.00  5.08
ATOM    162  CD  ARG A  26      18.069  -7.224  10.382  1.00  6.73
ATOM    163  NE  ARG A  26      17.000  -8.053   9.708  1.00  9.04
ATOM    164  CZ  ARG A  26      15.724  -8.206   9.912  1.00  7.06
ATOM    165  NH1 ARG A  26      15.085  -7.535  10.895  1.00 22.93
ATOM    166  NH2 ARG A  26      14.809  -8.825   9.346  1.00  7.89
ATOM    167  N   GLY A  27      18.761  -2.539   9.092  1.00  7.71
ATOM    168  CA  GLY A  27      18.537  -1.888   7.782  1.00  5.34
ATOM    169  C   GLY A  27      18.063  -2.896   6.862  1.00  4.70
ATOM    170  O   GLY A  27      18.155  -4.139   7.075  1.00 13.14
ATOM    171  N   SER A  28      17.562  -2.612   5.765  1.00 11.82
ATOM    172  CA  SER A  28      17.108  -3.325   4.615  1.00 14.72
ATOM    173  C   SER A  28      18.214  -4.327   4.142  1.00  7.74
ATOM    174  O   SER A  28      19.286  -3.973   4.083  1.00  6.71
ATOM    175  CB  SER A  28      16.460  -2.352   3.538  1.00  6.38
ATOM    176  OG  SER A  28      16.819  -0.978   3.833  1.00 28.10
ATOM    177  N   THR A  29      17.942  -5.634   4.241  1.00  4.79
ATOM    178  CA  THR A  29      18.562  -6.763   3.914  1.00  8.71
ATOM    179  C   THR A  29      19.500  -7.271   4.985  1.00 14.00
ATOM    180  O   THR A  29      20.162  -8.326   4.713  1.00 17.68
ATOM    181  CB  THR A  29      19.454  -6.680   2.617  1.00 14.90
ATOM    182  OG1 THR A  29      20.736  -6.066   2.595  1.00 14.00
ATOM    183  CG2 THR A  29      18.785  -5.888   1.561  1.00 15.59
ATOM    184  N   GLU A  30      19.740  -6.599   6.105  1.00 14.52
ATOM    185  CA  GLU A  30      20.677  -7.266   7.056  1.00 14.10
ATOM    186  C   GLU A  30      20.092  -8.513   7.647  1.00 13.07
ATOM    187  O   GLU A  30      18.916  -8.726   7.705  1.00 19.98
ATOM    188  CB  GLU A  30      21.228  -6.371   8.072  1.00 15.45
ATOM    189  CG  GLU A  30      21.166  -4.945   7.709  1.00  8.37
ATOM    190  CD  GLU A  30      22.073  -4.143   8.637  1.00 23.08
ATOM    191  OE1 GLU A  30      21.395  -3.328   9.284  1.00 19.26
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ATOM    192  OE2 GLU A  30      23.317  -4.327   8.712  1.00 19.71
ATOM    193  N   PRO A  31      20.875  -9.479   7.918  1.00 13.09
ATOM    194  CA  PRO A  31      20.477 -10.818   8.402  1.00 14.56
ATOM    195  C   PRO A  31      20.167 -10.698   9.895  1.00 18.27
ATOM    196  O   PRO A  31      20.148  -9.636  10.392  1.00 20.45
ATOM    197  CB  PRO A  31      21.690 -11.692   8.215  1.00 10.95
ATOM    198  CG  PRO A  31      22.790 -10.664   8.455  1.00 11.24
ATOM    199  CD  PRO A  31      22.350  -9.316   7.864  1.00 13.71
ATOM    200  N   GLY A  32      19.612 -11.689  10.472  1.00 18.99
ATOM    201  CA  GLY A  32      19.205 -11.774  11.816  1.00 13.53
ATOM    202  C   GLY A  32      18.133 -10.808  12.188  1.00 16.62
ATOM    203  O   GLY A  32      17.345 -10.294  11.411  1.00 17.01
ATOM    204  N   ASN A  33      18.055 -10.528  13.468  1.00 16.15
ATOM    205  CA  ASN A  33      17.290  -9.346  13.823  1.00 14.74
ATOM    206  C   ASN A  33      18.294  -8.273  14.230  1.00 15.46
ATOM    207  O   ASN A  33      17.774  -7.184  14.575  1.00 15.90
ATOM    208  CB  ASN A  33      16.241  -9.663  14.867  1.00 17.42
ATOM    209  CG  ASN A  33      16.827 -10.201  16.127  1.00 17.97
ATOM    210  OD1 ASN A  33      16.112 -10.395  17.089  1.00 19.05
ATOM    211  ND2 ASN A  33      18.074 -10.460  16.112  1.00 13.29
ATOM    212  N   MET A  34      19.633  -8.378  14.282  1.00 14.22
ATOM    213  CA  MET A  34      20.282  -7.171  14.751  1.00 12.97
ATOM    214  C   MET A  34      21.142  -6.663  13.611  1.00 19.02
ATOM    215  O   MET A  34      21.654  -5.512  13.713  1.00 26.04
ATOM    216  CB  MET A  34      21.202  -7.329  15.859  1.00 13.39
ATOM    217  CG  MET A  34      20.579  -7.713  17.163  1.00  9.02
ATOM    218  SD  MET A  34      20.175  -6.316  18.069  1.00  9.13
ATOM    219  CE  MET A  34      21.481  -5.121  18.095  1.00  4.11
ATOM    220  N   GLY A  35      21.259  -7.446  12.550  1.00 19.99
ATOM    221  CA  GLY A  35      22.071  -7.135  11.418  1.00 14.30
ATOM    222  C   GLY A  35      23.511  -7.340  11.764  1.00 17.58
ATOM    223  O   GLY A  35      23.965  -7.724  12.842  1.00 12.78
ATOM    224  N   ILE A  36      24.450  -6.839  10.950  1.00 20.63
ATOM    225  CA  ILE A  36      25.833  -7.029  11.277  1.00 17.71
ATOM    226  C   ILE A  36      26.609  -5.714  11.280  1.00 16.15
ATOM    227  O   ILE A  36      27.865  -5.618  11.662  1.00 20.30
ATOM    228  CB  ILE A  36      26.412  -8.070  10.327  1.00 30.19
ATOM    229  CG1 ILE A  36      26.088  -7.448   8.959  1.00 31.16
ATOM    230  CG2 ILE A  36      25.944  -9.490  10.543  1.00 15.68
ATOM    231  CD1 ILE A  36      26.922  -8.149   7.958  1.00 34.10
ATOM    232  N   THR A  37      25.905  -4.589  11.040  1.00 13.00
ATOM    233  CA  THR A  37      26.825  -3.396  11.141  1.00  9.67
ATOM    234  C   THR A  37      26.587  -2.513  12.350  1.00 15.44
ATOM    235  O   THR A  37      27.040  -3.055  13.410  1.00 20.20
ATOM    236  CB  THR A  37      26.592  -2.679   9.818  1.00 14.13
ATOM    237  OG1 THR A  37      25.241  -2.212   9.503  1.00 22.62
ATOM    238  CG2 THR A  37      26.949  -3.739   8.800  1.00  2.29
ATOM    239  N   VAL A  38      25.733  -1.493  12.249  1.00 11.92
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ATOM    240  CA  VAL A  38      25.237  -0.800  13.411  1.00 15.22
ATOM    241  C   VAL A  38      24.588  -1.455  14.612  1.00 14.68
ATOM    242  O   VAL A  38      24.906  -1.185  15.733  1.00 15.89
ATOM    243  CB  VAL A  38      24.124   0.180  12.855  1.00 14.13
ATOM    244  CG1 VAL A  38      23.663   0.897  14.167  1.00 13.55
ATOM    245  CG2 VAL A  38      24.570   1.025  11.670  1.00  6.75
ATOM    246  N   GLY A  39      23.745  -2.410  14.677  1.00 14.24
ATOM    247  CA  GLY A  39      23.135  -3.151  15.746  1.00 11.03
ATOM    248  C   GLY A  39      24.096  -3.586  16.791  1.00 13.34
ATOM    249  O   GLY A  39      24.131  -3.181  17.934  1.00 15.13
ATOM    250  N   PRO A  40      25.067  -4.340  16.352  1.00 14.70
ATOM    251  CA  PRO A  40      26.094  -5.025  17.171  1.00 13.44
ATOM    252  C   PRO A  40      27.010  -3.909  17.589  1.00 11.81
ATOM    253  O   PRO A  40      27.346  -3.871  18.764  1.00 12.79
ATOM    254  CB  PRO A  40      26.723  -6.111  16.279  1.00  8.43
ATOM    255  CG  PRO A  40      25.873  -6.243  14.950  1.00  4.84
ATOM    256  CD  PRO A  40      25.198  -4.902  14.995  1.00 12.36
ATOM    257  N   ALA A  41      27.226  -2.979  16.695  1.00  7.41
ATOM    258  CA  ALA A  41      28.066  -1.962  17.278  1.00 11.03
ATOM    259  C   ALA A  41      27.378  -1.206  18.439  1.00 14.87
ATOM    260  O   ALA A  41      28.028  -0.503  19.274  1.00 14.26
ATOM    261  CB  ALA A  41      28.579  -0.905  16.313  1.00  7.17
ATOM    262  N   LEU A  42      26.135  -0.811  18.237  1.00 11.87
ATOM    263  CA  LEU A  42      25.487  -0.048  19.300  1.00 12.36
ATOM    264  C   LEU A  42      25.337  -0.856  20.624  1.00 11.94
ATOM    265  O   LEU A  42      25.423  -0.397  21.730  1.00  8.33
ATOM    266  CB  LEU A  42      24.036   0.168  18.811  1.00 13.24
ATOM    267  CG  LEU A  42      23.272   1.160  19.676  1.00  6.90
ATOM    268  CD1 LEU A  42      24.108   2.419  19.962  1.00  6.62
ATOM    269  CD2 LEU A  42      21.991   1.580  18.943  1.00  7.11
ATOM    270  N   ALA A  43      24.905  -2.095  20.482  1.00 10.88
ATOM    271  CA  ALA A  43      24.761  -3.027  21.553  1.00 12.37
ATOM    272  C   ALA A  43      26.106  -3.136  22.252  1.00 15.45
ATOM    273  O   ALA A  43      25.958  -2.743  23.433  1.00 20.80
ATOM    274  CB  ALA A  43      24.148  -4.324  21.002  1.00  9.60
ATOM    275  N   ASN A  44      27.263  -3.440  21.636  1.00 16.91
ATOM    276  CA  ASN A  44      28.454  -3.434  22.439  1.00 20.33
ATOM    277  C   ASN A  44      28.717  -2.044  23.113  1.00 17.66
ATOM    278  O   ASN A  44      29.019  -1.991  24.301  1.00 17.06
ATOM    279  CB  ASN A  44      29.756  -3.695  21.625  1.00 35.48
ATOM    280  CG  ASN A  44      29.564  -5.115  21.138  1.00 58.23
ATOM    281  OD1 ASN A  44      30.013  -5.403  20.034  1.00 79.77
ATOM    282  ND2 ASN A  44      28.908  -5.945  21.921  1.00 70.10
ATOM    283  N   GLY A  45      28.682  -0.988  22.297  1.00 14.39
ATOM    284  CA  GLY A  45      29.015   0.221  22.976  1.00 11.65
ATOM    285  C   GLY A  45      28.175   0.255  24.234  1.00 14.30
ATOM    286  O   GLY A  45      28.529   0.582  25.385  1.00 10.77
ATOM    287  N   LEU A  46      26.861   0.099  24.065  1.00 16.88
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ATOM    288  CA  LEU A  46      25.968   0.248  25.207  1.00 16.29
ATOM    289  C   LEU A  46      26.395  -0.651  26.346  1.00 13.48
ATOM    290  O   LEU A  46      26.579  -0.325  27.462  1.00  7.75
ATOM    291  CB  LEU A  46      24.608  -0.243  24.847  1.00 19.46
ATOM    292  CG  LEU A  46      23.642   0.551  25.664  1.00 13.97
ATOM    293  CD1 LEU A  46      24.089   1.994  25.563  1.00 13.99
ATOM    294  CD2 LEU A  46      22.275   0.465  25.038  1.00 32.18
ATOM    295  N   GLU A  47      26.523  -1.890  25.882  1.00 15.90
ATOM    296  CA  GLU A  47      26.910  -2.886  26.909  1.00 24.03
ATOM    297  C   GLU A  47      28.140  -2.500  27.702  1.00 24.14
ATOM    298  O   GLU A  47      28.722  -3.203  28.500  1.00 27.24
ATOM    299  CB  GLU A  47      27.147  -4.206  26.204  1.00 33.33
ATOM    300  CG  GLU A  47      27.386  -5.254  27.245  1.00 51.29
ATOM    301  CD  GLU A  47      27.661  -6.560  26.524  1.00 68.40
ATOM    302  OE1 GLU A  47      26.741  -7.007  25.777  1.00 66.37
ATOM    303  OE2 GLU A  47      28.856  -6.921  26.830  1.00 78.70
ATOM    304  N   SER A  48      28.992  -1.626  27.215  1.00 27.50
ATOM    305  CA  SER A  48      30.331  -1.518  27.789  1.00 25.23
ATOM    306  C   SER A  48      30.108  -0.555  28.926  1.00 26.91
ATOM    307  O   SER A  48      31.124  -0.058  29.462  1.00 33.39
ATOM    308  CB  SER A  48      31.116  -0.990  26.621  1.00 21.90
ATOM    309  OG  SER A  48      31.294   0.422  26.483  1.00 27.87
ATOM    310  N   HIS A  49      28.826  -0.101  28.995  1.00 25.04
ATOM    311  CA  HIS A  49      28.542   0.955  29.956  1.00 19.72
ATOM    312  C   HIS A  49      27.480   0.461  30.950  1.00 22.55
ATOM    313  O   HIS A  49      27.186   1.089  31.898  1.00 27.93
ATOM    314  CB  HIS A  49      28.094   2.197  29.463  1.00 16.13
ATOM    315  CG  HIS A  49      28.806   3.036  28.520  1.00 39.79
ATOM    316  ND1 HIS A  49      29.564   4.058  28.953  1.00 45.66
ATOM    317  CD2 HIS A  49      28.776   3.070  27.197  1.00 46.91
ATOM    318  CE1 HIS A  49      30.028   4.750  27.979  1.00 45.87
ATOM    319  NE2 HIS A  49      29.544   4.139  26.934  1.00 50.84
ATOM    320  N   ILE A  50      27.009  -0.703  30.715  1.00 18.34
ATOM    321  CA  ILE A  50      25.874  -1.129  31.415  1.00 19.89
ATOM    322  C   ILE A  50      25.917  -2.629  31.146  1.00 26.29
ATOM    323  O   ILE A  50      25.322  -3.023  30.168  1.00 25.33
ATOM    324  CB  ILE A  50      24.527  -0.535  31.008  1.00 10.50
ATOM    325  CG1 ILE A  50      24.340   0.906  31.292  1.00  4.97
ATOM    326  CG2 ILE A  50      23.466  -1.298  31.697  1.00 12.96
ATOM    327  CD1 ILE A  50      23.413   1.845  30.602  1.00 16.65
ATOM    328  N   ARG A  51      26.707  -3.256  32.066  1.00 31.77
ATOM    329  CA  ARG A  51      26.887  -4.714  32.107  1.00 29.06
ATOM    330  C   ARG A  51      25.457  -5.331  32.170  1.00 32.68
ATOM    331  O   ARG A  51      25.396  -6.363  31.512  1.00 37.16
ATOM    332  N   ASN A  52      24.380  -4.817  32.788  1.00 28.48
ATOM    333  CA  ASN A  52      23.284  -5.767  32.832  1.00 26.39
ATOM    334  C   ASN A  52      22.176  -5.178  31.993  1.00 27.75
ATOM    335  O   ASN A  52      21.333  -4.488  32.636  1.00 26.68
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ATOM    336  CB  ASN A  52      22.750  -5.884  34.232  1.00 34.86
ATOM    337  CG  ASN A  52      21.637  -6.879  34.271  1.00 39.54
ATOM    338  OD1 ASN A  52      20.781  -6.541  35.095  1.00 54.31
ATOM    339  ND2 ASN A  52      21.611  -7.954  33.503  1.00 48.82
ATOM    340  N   ILE A  53      22.127  -5.699  30.800  1.00 24.42
ATOM    341  CA  ILE A  53      21.261  -5.092  29.772  1.00 20.15
ATOM    342  C   ILE A  53      20.585  -6.151  28.912  1.00 17.63
ATOM    343  O   ILE A  53      21.020  -7.349  28.917  1.00 18.01
ATOM    344  CB  ILE A  53      22.245  -4.297  28.880  1.00 14.09
ATOM    345  CG1 ILE A  53      21.682  -3.257  27.936  1.00 22.91
ATOM    346  CG2 ILE A  53      22.907  -5.321  27.946  1.00 16.37
ATOM    347  CD1 ILE A  53      22.877  -2.315  27.622  1.00 38.17
ATOM    348  N   TRP A  54      19.447  -5.880  28.383  1.00 15.19
ATOM    349  CA  TRP A  54      18.804  -6.889  27.567  1.00 17.96
ATOM    350  C   TRP A  54      18.803  -6.230  26.151  1.00 19.82
ATOM    351  O   TRP A  54      18.340  -5.059  25.985  1.00 18.37
ATOM    352  CB  TRP A  54      17.364  -7.046  27.998  1.00 23.18
ATOM    353  CG  TRP A  54      16.949  -7.932  29.100  1.00 24.57
ATOM    354  CD1 TRP A  54      17.757  -8.727  29.895  1.00 24.46
ATOM    355  CD2 TRP A  54      15.595  -8.164  29.603  1.00 30.21
ATOM    356  NE1 TRP A  54      17.004  -9.372  30.858  1.00 25.87
ATOM    357  CE2 TRP A  54      15.692  -9.039  30.700  1.00 24.92
ATOM    358  CE3 TRP A  54      14.358  -7.633  29.243  1.00 36.26
ATOM    359  CZ2 TRP A  54      14.611  -9.442  31.432  1.00 19.75
ATOM    360  CZ3 TRP A  54      13.316  -8.042  30.009  1.00 32.94
ATOM    361  CH2 TRP A  54      13.451  -8.916  31.068  1.00 23.02
ATOM    362  N   ILE A  55      19.063  -7.152  25.204  1.00 15.21
ATOM    363  CA  ILE A  55      19.178  -6.655  23.838  1.00 12.41
ATOM    364  C   ILE A  55      18.091  -7.215  22.962  1.00 11.40
ATOM    365  O   ILE A  55      17.955  -8.378  22.680  1.00  7.34
ATOM    366  CB  ILE A  55      20.546  -6.962  23.201  1.00 16.44
ATOM    367  CG1 ILE A  55      21.939  -6.409  23.702  1.00  8.75
ATOM    368  CG2 ILE A  55      20.384  -6.460  21.750  1.00 21.77
ATOM    369  CD1 ILE A  55      21.767  -5.582  24.863  1.00 16.23
ATOM    370  N   GLN A  56      17.226  -6.412  22.390  1.00  9.67
ATOM    371  CA  GLN A  56      16.161  -7.016  21.619  1.00 10.90
ATOM    372  C   GLN A  56      16.432  -6.621  20.143  1.00 13.08
ATOM    373  O   GLN A  56      16.402  -5.393  19.953  1.00 10.32
ATOM    374  CB  GLN A  56      14.786  -6.542  22.014  1.00 11.49
ATOM    375  CG  GLN A  56      13.653  -7.256  21.316  1.00 23.47
ATOM    376  CD  GLN A  56      13.789  -8.741  21.351  1.00 24.88
ATOM    377  OE1 GLN A  56      13.610  -9.379  20.324  1.00  9.56
ATOM    378  NE2 GLN A  56      14.119  -9.221  22.544  1.00 17.94
ATOM    379  N   GLY A  57      16.288  -7.645  19.216  1.00  6.84
ATOM    380  CA  GLY A  57      16.174  -7.019  17.841  1.00 16.15
ATOM    381  C   GLY A  57      14.740  -7.085  17.267  1.00 13.72
ATOM    382  O   GLY A  57      14.124  -8.016  17.752  1.00 12.70
ATOM    383  N   VAL A  58      14.068  -6.264  16.525  1.00 12.73
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ATOM	384	CA	VAL	A	58	12.739	-6.308	16.070	1.00	11.16
ATOM	385	C	VAL	A	58	12.715	-7.246	14.893	1.00	14.85
ATOM	386	O	VAL	A	58	13.234	-6.891	13.849	1.00	18.64
ATOM	387	CB	VAL	A	58	12.262	-4.984	15.352	1.00	6.54
ATOM	388	CG1	VAL	A	58	10.894	-4.974	14.731	1.00	5.89
ATOM	389	CG2	VAL	A	58	12.650	-3.840	16.331	1.00	5.86
ATOM	390	N	GLY	A	59	12.209	-8.465	15.008	1.00	21.96
ATOM	391	CA	GLY	A	59	12.120	-9.385	13.874	1.00	17.81
ATOM	392	C	GLY	A	59	10.645	-9.561	13.550	1.00	23.35
ATOM	393	O	GLY	A	59	9.919	-8.579	13.249	1.00	27.99
ATOM	394	N	GLY	A	60	10.166	-10.805	13.623	1.00	18.75
ATOM	395	CA	GLY	A	60	8.841	-11.142	13.285	1.00	11.46
ATOM	396	C	GLY	A	60	8.550	-10.833	11.851	1.00	14.56
ATOM	397	O	GLY	A	60	9.160	-11.439	11.003	1.00	16.32
ATOM	398	N	PRO	A	61	7.505	-10.103	11.612	1.00	12.10
ATOM	399	CA	PRO	A	61	7.123	-9.774	10.250	1.00	14.70
ATOM	400	C	PRO	A	61	8.230	-8.941	9.570	1.00	22.17
ATOM	401	O	PRO	A	61	8.143	-8.758	8.344	1.00	25.74
ATOM	402	CB	PRO	A	61	5.911	-8.860	10.332	1.00	14.30
ATOM	403	CG	PRO	A	61	5.880	-8.514	11.784	1.00	13.62
ATOM	404	CD	PRO	A	61	6.723	-9.417	12.576	1.00	12.29
ATOM	405	N	TYR	A	62	9.162	-8.257	10.292	1.00	21.56
ATOM	406	CA	TYR	A	62	9.973	-7.242	9.674	1.00	17.07
ATOM	407	C	TYR	A	62	11.133	-7.907	9.047	1.00	18.73
ATOM	408	O	TYR	A	62	12.132	-8.213	9.691	1.00	22.39
ATOM	409	CB	TYR	A	62	10.504	-6.401	10.803	1.00	17.51
ATOM	410	CG	TYR	A	62	11.461	-5.421	10.236	1.00	15.23
ATOM	411	CD1	TYR	A	62	11.343	-4.920	9.032	1.00	17.79
ATOM	412	CD2	TYR	A	62	12.465	-4.971	10.969	1.00	19.09
ATOM	413	CE1	TYR	A	62	12.206	-3.997	8.506	1.00	19.28
ATOM	414	CE2	TYR	A	62	13.438	-4.101	10.490	1.00	25.40
ATOM	415	CZ	TYR	A	62	13.327	-3.571	9.186	1.00	20.95
ATOM	416	OH	TYR	A	62	14.320	-2.649	8.791	1.00	14.70
ATOM	417	N	ASP	A	63	10.998	-8.419	7.816	1.00	19.47
ATOM	418	CA	ASP	A	63	12.137	-9.011	7.081	1.00	17.52
ATOM	419	C	ASP	A	63	13.027	-7.973	6.453	1.00	17.97
ATOM	420	O	ASP	A	63	13.628	-8.442	5.512	1.00	14.94
ATOM	421	CB	ASP	A	63	11.474	-9.873	6.015	1.00	17.16
ATOM	422	CG	ASP	A	63	10.563	-9.136	5.096	1.00	27.75
ATOM	423	OD1	ASP	A	63	10.049	-8.030	5.281	1.00	34.11
ATOM	424	OD2	ASP	A	63	10.300	-9.635	4.002	1.00	44.13
ATOM	425	N	ALA	A	64	13.089	-6.685	6.584	1.00	15.36
ATOM	426	CA	ALA	A	64	14.054	-5.725	6.098	1.00	17.14
ATOM	427	C	ALA	A	64	14.118	-5.780	4.589	1.00	21.10
ATOM	428	O	ALA	A	64	15.193	-5.861	3.968	1.00	23.12
ATOM	429	CB	ALA	A	64	15.458	-5.861	6.646	1.00	20.45
ATOM	430	N	ALA	A	65	12.946	-6.009	4.006	1.00	22.21
ATOM	431	CA	ALA	A	65	12.817	-6.072	2.565	1.00	21.81

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ATOM    432  C   ALA A  65      13.143  -4.857   1.745  1.00 21.76
ATOM    433  O   ALA A  65      12.855  -3.801   2.229  1.00 23.60
ATOM    434  CB  ALA A  65      11.384  -6.390   2.364  1.00 17.31
ATOM    435  N   LEU A  66      13.401  -4.866   0.402  1.00 21.48
ATOM    436  CA  LEU A  66      13.763  -3.581  -0.216  1.00 13.20
ATOM    437  C   LEU A  66      12.469  -2.913  -0.452  1.00 13.90
ATOM    438  O   LEU A  66      12.548  -1.767  -0.197  1.00 11.85
ATOM    439  CB  LEU A  66      14.593  -3.602  -1.470  1.00  3.92
ATOM    440  CG  LEU A  66      15.891  -4.308  -1.191  1.00  9.05
ATOM    441  CD1 LEU A  66      16.509  -4.725  -2.438  1.00 12.78
ATOM    442  CD2 LEU A  66      16.569  -3.119  -0.580  1.00 13.44
ATOM    443  N   ALA A  67      11.413  -3.625  -0.801  1.00 14.94
ATOM    444  CA  ALA A  67      10.253  -2.759  -1.277  1.00 12.42
ATOM    445  C   ALA A  67       9.626  -1.879  -0.224  1.00 14.21
ATOM    446  O   ALA A  67       9.218  -0.818  -0.643  1.00 14.29
ATOM    447  CB  ALA A  67       9.089  -3.588  -1.781  1.00  3.90
ATOM    448  N   THR A  68       9.494  -2.409   1.006  1.00 12.11
ATOM    449  CA  THR A  68       8.780  -1.647   1.997  1.00 11.77
ATOM    450  C   THR A  68       9.242  -0.214   2.219  1.00 13.05
ATOM    451  O   THR A  68       8.597   0.683   2.766  1.00 11.13
ATOM    452  CB  THR A  68       8.892  -2.488   3.241  1.00 13.93
ATOM    453  OG1 THR A  68      10.145  -3.150   3.224  1.00 27.44
ATOM    454  CG2 THR A  68       7.783  -3.459   3.087  1.00 13.39
ATOM    455  N   ASN A  69      10.450  -0.057   1.808  1.00  7.59
ATOM    456  CA  ASN A  69      11.020   1.236   1.791  1.00  8.76
ATOM    457  C   ASN A  69      10.095   2.165   1.047  1.00 10.28
ATOM    458  O   ASN A  69       9.950   3.345   1.305  1.00  5.30
ATOM    459  CB  ASN A  69      12.461   1.251   1.231  1.00  5.54
ATOM    460  CG  ASN A  69      13.374   1.207   2.398  1.00 15.08
ATOM    461  OD1 ASN A  69      13.307   2.124   3.275  1.00 31.90
ATOM    462  ND2 ASN A  69      14.048   0.099   2.360  1.00  4.51
ATOM    463  N   PHE A  70       9.390   1.656   0.079  1.00 19.09
ATOM    464  CA  PHE A  70       8.552   2.619  -0.631  1.00 21.80
ATOM    465  C   PHE A  70       7.157   2.836  -0.123  1.00 23.36
ATOM    466  O   PHE A  70       6.509   3.717  -0.724  1.00 25.74
ATOM    467  CB  PHE A  70       8.547   2.386  -2.082  1.00 17.38
ATOM    468  CG  PHE A  70       9.870   2.360  -2.770  1.00 15.72
ATOM    469  CD1 PHE A  70      10.080   3.430  -3.576  1.00  5.15
ATOM    470  CD2 PHE A  70      10.702   1.245  -2.497  1.00  7.61
ATOM    471  CE1 PHE A  70      11.268   3.330  -4.191  1.00 16.05
ATOM    472  CE2 PHE A  70      11.913   1.267  -3.168  1.00 22.23
ATOM    473  CZ  PHE A  70      12.199   2.314  -4.016  1.00  9.57
ATOM    474  N   LEU A  71       6.765   2.246   1.034  1.00 25.53
ATOM    475  CA  LEU A  71       5.506   2.725   1.599  1.00 24.24
ATOM    476  C   LEU A  71       5.649   4.037   2.343  1.00 27.91
ATOM    477  O   LEU A  71       6.694   4.521   2.750  1.00 28.86
ATOM    478  CB  LEU A  71       5.150   1.635   2.535  1.00 19.99
ATOM    479  CG  LEU A  71       5.003   0.342   1.873  1.00 16.09
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ATOM    480  CD1 LEU A  71      4.879  -0.764   2.885  1.00 18.12
ATOM    481  CD2 LEU A  71      3.786   0.546   1.000  1.00 18.24
ATOM    482  N   PRO A  72      4.535   4.663   2.529  1.00 33.01
ATOM    483  CA  PRO A  72      4.389   5.888   3.311  1.00 34.96
ATOM    484  C   PRO A  72      4.865   5.590   4.778  1.00 32.90
ATOM    485  O   PRO A  72      4.619   4.512   5.331  1.00 28.55
ATOM    486  CB  PRO A  72      2.983   6.453   3.095  1.00 32.98
ATOM    487  CG  PRO A  72      2.224   5.189   2.827  1.00 30.36
ATOM    488  CD  PRO A  72      3.188   4.093   2.380  1.00 33.56
ATOM    489  N   ARG A  73      5.601   6.610   5.221  1.00 27.54
ATOM    490  CA  ARG A  73      6.325   6.547   6.408  1.00 25.42
ATOM    491  C   ARG A  73      7.613   5.755   6.321  1.00 21.78
ATOM    492  O   ARG A  73      8.360   5.950   7.304  1.00 29.61
ATOM    493  CB  ARG A  73      5.469   5.978   7.549  1.00 24.29
ATOM    494  CG  ARG A  73      4.575   6.998   8.155  1.00 23.47
ATOM    495  CD  ARG A  73      3.818   6.793   9.360  1.00 29.73
ATOM    496  NE  ARG A  73      3.222   5.460   9.392  1.00 36.30
ATOM    497  CZ  ARG A  73      2.891   5.312  10.713  1.00 42.26
ATOM    498  NH1 ARG A  73      3.145   6.288  11.555  1.00 26.57
ATOM    499  NH2 ARG A  73      2.320   4.144  10.883  1.00 39.03
ATOM    500  N   GLY A  74      7.868   4.909   5.326  1.00  8.42
ATOM    501  CA  GLY A  74      9.120   4.291   5.332  1.00  5.06
ATOM    502  C   GLY A  74      9.243   2.858   5.508  1.00 12.74
ATOM    503  O   GLY A  74     10.256   2.286   5.317  1.00 16.46
ATOM    504  N   THR A  75      8.145   2.321   5.906  1.00 12.82
ATOM    505  CA  THR A  75      8.036   0.869   6.008  1.00 11.14
ATOM    506  C   THR A  75      6.625   0.428   6.134  1.00 10.64
ATOM    507  O   THR A  75      5.757   1.231   5.949  1.00  9.36
ATOM    508  CB  THR A  75      8.843   0.398   7.219  1.00  6.97
ATOM    509  OG1 THR A  75      8.938  -0.950   7.125  1.00  5.64
ATOM    510  CG2 THR A  75      8.108   0.865   8.603  1.00  6.30
ATOM    511  N   SER A  76      6.409  -0.858   6.259  1.00 10.07
ATOM    512  CA  SER A  76      5.061  -1.384   6.354  1.00 13.33
ATOM    513  C   SER A  76      4.405  -1.163   7.747  1.00 21.87
ATOM    514  O   SER A  76      5.228  -1.102   8.679  1.00 24.22
ATOM    515  CB  SER A  76      5.030  -2.832   6.083  1.00  4.81
ATOM    516  OG  SER A  76      5.327  -3.664   7.107  1.00 16.98
ATOM    517  N   GLN A  77      3.082  -1.100   7.911  1.00 24.90
ATOM    518  CA  GLN A  77      2.454  -1.020   9.166  1.00 23.85
ATOM    519  C   GLN A  77      2.643  -2.236  10.015  1.00 19.58
ATOM    520  O   GLN A  77      2.908  -2.140  11.203  1.00 15.15
ATOM    521  CB  GLN A  77      0.983  -0.703   9.217  1.00 32.64
ATOM    522  CG  GLN A  77      0.567  -0.580  10.642  1.00 49.56
ATOM    523  CD  GLN A  77      0.689   0.785  11.194  1.00 65.91
ATOM    524  OE1 GLN A  77      0.956   0.869  12.356  1.00 66.06
ATOM    525  NE2 GLN A  77      0.481   1.750  10.350  1.00 68.91
ATOM    526  N   ALA A  78      2.754  -3.376   9.402  1.00 15.90
ATOM    527  CA  ALA A  78      3.071  -4.577  10.073  1.00 19.47
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ATOM    528  C   ALA A  78      4.381  -4.332  10.819  1.00 24.48
ATOM    529  O   ALA A  78      4.389  -4.729  11.983  1.00 26.91
ATOM    530  CB  ALA A  78      3.390  -5.808   9.336  1.00 17.23
ATOM    531  N   ASN A  79      5.350  -3.863  10.093  1.00 21.58
ATOM    532  CA  ASN A  79      6.602  -3.576  10.774  1.00 20.62
ATOM    533  C   ASN A  79      6.480  -2.673  11.969  1.00 20.93
ATOM    534  O   ASN A  79      6.975  -2.944  13.053  1.00 15.52
ATOM    535  CB  ASN A  79      7.474  -3.069   9.670  1.00 24.79
ATOM    536  CG  ASN A  79      7.933  -4.238   8.824  1.00 28.76
ATOM    537  OD1 ASN A  79      7.867  -5.439   9.091  1.00 25.30
ATOM    538  ND2 ASN A  79      8.488  -3.891   7.660  1.00 24.90
ATOM    539  N   ILE A  80      5.731  -1.611  11.936  1.00 15.93
ATOM    540  CA  ILE A  80      5.586  -0.574  12.924  1.00 17.00
ATOM    541  C   ILE A  80      4.925  -1.187  14.118  1.00 20.63
ATOM    542  O   ILE A  80      5.234  -0.939  15.264  1.00 18.79
ATOM    543  CB  ILE A  80      4.756   0.629  12.436  1.00 11.98
ATOM    544  CG1 ILE A  80      5.627   1.124  11.297  1.00  9.50
ATOM    545  CG2 ILE A  80      4.379   1.728  13.354  1.00 16.27
ATOM    546  CD1 ILE A  80      5.007   2.071  10.424  1.00  8.15
ATOM    547  N   ASP A  81      4.017  -2.019  13.708  1.00 19.21
ATOM    548  CA  ASP A  81      3.304  -2.778  14.728  1.00 15.15
ATOM    549  C   ASP A  81      4.147  -3.711  15.510  1.00 15.77
ATOM    550  O   ASP A  81      4.084  -3.697  16.695  1.00 15.82
ATOM    551  CB  ASP A  81      2.291  -3.438  13.868  1.00 26.36
ATOM    552  CG  ASP A  81      1.065  -2.530  13.790  1.00 23.71
ATOM    553  OD1 ASP A  81      1.105  -1.355  14.226  1.00 14.33
ATOM    554  OD2 ASP A  81      0.061  -3.125  13.222  1.00 33.05
ATOM    555  N   GLU A  82      5.148  -4.447  15.096  1.00 16.07
ATOM    556  CA  GLU A  82      5.984  -5.318  15.882  1.00 14.77
ATOM    557  C   GLU A  82      6.839  -4.355  16.667  1.00 19.33
ATOM    558  O   GLU A  82      7.315  -4.708  17.752  1.00 23.58
ATOM    559  CB  GLU A  82      6.998  -6.031  15.064  1.00 13.20
ATOM    560  CG  GLU A  82      7.792  -7.239  15.476  1.00 23.09
ATOM    561  CD  GLU A  82      6.767  -8.114  16.185  1.00 29.68
ATOM    562  OE1 GLU A  82      5.666  -7.670  16.403  1.00 26.63
ATOM    563  OE2 GLU A  82      7.273  -9.181  16.411  1.00 33.08
ATOM    564  N   GLY A  83      7.228  -3.227  16.199  1.00 16.79
ATOM    565  CA  GLY A  83      8.033  -2.428  17.140  1.00 17.32
ATOM    566  C   GLY A  83      7.238  -2.018  18.366  1.00 17.54
ATOM    567  O   GLY A  83      7.561  -2.103  19.528  1.00 15.06
ATOM    568  N   LYS A  84      6.093  -1.408  18.114  1.00 18.72
ATOM    569  CA  LYS A  84      5.050  -1.146  19.096  1.00 16.90
ATOM    570  C   LYS A  84      4.893  -2.337  20.057  1.00 17.74
ATOM    571  O   LYS A  84      4.962  -2.265  21.295  1.00 14.31
ATOM    572  CB  LYS A  84      3.799  -0.872  18.307  1.00 14.62
ATOM    573  CG  LYS A  84      3.535   0.565  18.291  1.00 19.30
ATOM    574  CD  LYS A  84      2.787   1.013  17.044  1.00 34.24
ATOM    575  CE  LYS A  84      1.568   1.902  17.337  1.00 37.70
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ATOM    576  NZ  LYS A  84       0.346   1.226  16.827  1.00 48.42
ATOM    577  N   ARG A  85       4.617  -3.506  19.519  1.00 18.50
ATOM    578  CA  ARG A  85       4.583  -4.705  20.280  1.00 19.04
ATOM    579  C   ARG A  85       5.677  -4.733  21.308  1.00 19.63
ATOM    580  O   ARG A  85       5.442  -5.192  22.383  1.00 19.24
ATOM    581  CB  ARG A  85       4.740  -5.979  19.464  1.00 14.74
ATOM    582  CG  ARG A  85       3.843  -7.094  19.887  1.00  8.85
ATOM    583  CD  ARG A  85       4.146  -8.554  19.705  1.00  7.20
ATOM    584  NE  ARG A  85       5.483  -8.898  19.194  1.00 20.30
ATOM    585  CZ  ARG A  85       6.170  -9.705  19.899  1.00 18.19
ATOM    586  NH1 ARG A  85       5.627 -10.161  21.040  1.00 34.03
ATOM    587  NH2 ARG A  85       7.345  -9.979  19.555  1.00 15.36
ATOM    588  N   LEU A  86       6.901  -4.586  20.956  1.00 22.21
ATOM    589  CA  LEU A  86       8.006  -4.792  21.873  1.00 20.94
ATOM    590  C   LEU A  86       8.044  -3.637  22.803  1.00 20.73
ATOM    591  O   LEU A  86       8.155  -3.970  23.925  1.00 22.18
ATOM    592  CB  LEU A  86       9.333  -4.932  21.168  1.00  6.67
ATOM    593  CG  LEU A  86       9.358  -6.241  20.282  1.00 11.45
ATOM    594  CD1 LEU A  86      10.546  -6.054  19.287  1.00 18.60
ATOM    595  CD2 LEU A  86       9.362  -7.516  21.020  1.00  5.17
ATOM    596  N   PHE A  87       7.700  -2.446  22.529  1.00 16.79
ATOM    597  CA  PHE A  87       7.850  -1.416  23.492  1.00 18.21
ATOM    598  C   PHE A  87       6.939  -1.805  24.618  1.00 26.51
ATOM    599  O   PHE A  87       7.082  -1.565  25.839  1.00 30.36
ATOM    600  CB  PHE A  87       7.498  -0.118  22.846  1.00 15.81
ATOM    601  CG  PHE A  87       8.661   0.503  22.128  1.00 22.72
ATOM    602  CD1 PHE A  87       9.625   1.163  22.795  1.00 25.90
ATOM    603  CD2 PHE A  87       8.800   0.446  20.774  1.00 24.19
ATOM    604  CE1 PHE A  87      10.699   1.781  22.220  1.00 26.46
ATOM    605  CE2 PHE A  87       9.871   0.991  20.153  1.00 29.24
ATOM    606  CZ  PHE A  87      10.827   1.669  20.849  1.00 20.81
ATOM    607  N   ALA A  88       5.862  -2.422  24.266  1.00 29.15
ATOM    608  CA  ALA A  88       4.772  -2.699  25.195  1.00 22.92
ATOM    609  C   ALA A  88       5.186  -3.837  26.068  1.00 22.03
ATOM    610  O   ALA A  88       4.974  -3.879  27.284  1.00 27.02
ATOM    611  CB  ALA A  88       3.551  -2.803  24.299  1.00 22.13
ATOM    612  N   LEU A  89       5.649  -4.897  25.531  1.00 19.16
ATOM    613  CA  LEU A  89       6.188  -6.032  26.208  1.00 19.29
ATOM    614  C   LEU A  89       7.250  -5.507  27.133  1.00 22.06
ATOM    615  O   LEU A  89       7.449  -6.050  28.177  1.00 20.49
ATOM    616  CB  LEU A  89       7.021  -6.863  25.221  1.00 18.41
ATOM    617  CG  LEU A  89       7.477  -8.167  25.834  1.00 20.45
ATOM    618  CD1 LEU A  89       6.326  -8.707  26.627  1.00 17.22
ATOM    619  CD2 LEU A  89       8.060  -9.057  24.769  1.00 18.83
ATOM    620  N   ALA A  90       8.124  -4.644  26.722  1.00 22.80
ATOM    621  CA  ALA A  90       9.027  -4.137  27.701  1.00 24.14
ATOM    622  C   ALA A  90       8.237  -3.488  28.849  1.00 23.63
ATOM    623  O   ALA A  90       8.414  -3.835  30.071  1.00 22.73
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ATOM    624  CB  ALA A  90      10.080  -3.253  27.139  1.00  7.74
ATOM    625  N   ASN A  91       7.457  -2.445  28.732  1.00 25.45
ATOM    626  CA  ASN A  91       6.665  -1.979  29.870  1.00 27.25
ATOM    627  C   ASN A  91       5.847  -2.996  30.656  1.00 30.97
ATOM    628  O   ASN A  91       5.346  -2.884  31.768  1.00 27.64
ATOM    629  CB  ASN A  91       5.560  -1.206  29.125  1.00 29.14
ATOM    630  CG  ASN A  91       4.946  -0.345  30.216  1.00 31.73
ATOM    631  OD1 ASN A  91       3.845  -0.692  30.645  1.00 46.76
ATOM    632  ND2 ASN A  91       5.641   0.629  30.643  1.00 29.03
ATOM    633  N   GLN A  92       5.369  -4.008  29.969  1.00 35.37
ATOM    634  CA  GLN A  92       4.702  -5.141  30.591  1.00 35.55
ATOM    635  C   GLN A  92       5.619  -6.072  31.352  1.00 34.28
ATOM    636  O   GLN A  92       5.227  -6.519  32.440  1.00 39.47
ATOM    637  CB  GLN A  92       3.866  -5.903  29.573  1.00 54.94
ATOM    638  CG  GLN A  92       2.689  -6.698  30.142  1.00 78.63
ATOM    639  CD  GLN A  92       2.806  -8.167  29.805  1.00 93.87
ATOM    640  OE1 GLN A  92       3.597  -8.840  30.475  1.00 96.99
ATOM    641  NE2 GLN A  92       2.083  -8.696  28.824  1.00 97.81
ATOM    642  N   LYS A  93       6.859  -6.403  31.050  1.00 31.97
ATOM    643  CA  LYS A  93       7.675  -7.204  31.972  1.00 25.22
ATOM    644  C   LYS A  93       8.381  -6.298  33.015  1.00 24.68
ATOM    645  O   LYS A  93       8.716  -6.793  34.075  1.00 32.13
ATOM    646  CB  LYS A  93       8.673  -7.980  31.148  1.00 10.86
ATOM    647  CG  LYS A  93       8.225  -8.963  30.159  1.00 24.26
ATOM    648  CD  LYS A  93       9.362  -9.966  29.986  1.00 21.96
ATOM    649  CE  LYS A  93       9.093 -10.718  28.658  1.00 23.78
ATOM    650  NZ  LYS A  93      10.084 -11.805  28.300  1.00 25.87
ATOM    651  N   CYS A  94       8.752  -5.096  32.774  1.00 16.62
ATOM    652  CA  CYS A  94       9.752  -4.412  33.480  1.00 18.95
ATOM    653  C   CYS A  94       9.512  -2.936  33.537  1.00 24.83
ATOM    654  O   CYS A  94      10.184  -2.017  33.150  1.00 26.80
ATOM    655  CB  CYS A  94      11.147  -4.691  32.911  1.00  3.14
ATOM    656  SG  CYS A  94      11.618  -6.437  32.882  1.00 25.28
ATOM    657  N   PRO A  95       8.403  -2.561  34.086  1.00 26.08
ATOM    658  CA  PRO A  95       7.891  -1.202  33.878  1.00 26.11
ATOM    659  C   PRO A  95       8.960  -0.259  34.299  1.00 27.32
ATOM    660  O   PRO A  95       8.776   0.966  34.108  1.00 29.08
ATOM    661  CB  PRO A  95       6.609  -1.090  34.747  1.00 20.75
ATOM    662  CG  PRO A  95       6.587  -2.421  35.322  1.00 19.04
ATOM    663  CD  PRO A  95       7.363  -3.461  34.509  1.00 22.55
ATOM    664  N   ASN A  96       9.836  -0.776  35.193  1.00 31.44
ATOM    665  CA  ASN A  96      10.559   0.274  35.966  1.00 35.38
ATOM    666  C   ASN A  96      11.891   0.476  35.353  1.00 33.83
ATOM    667  O   ASN A  96      12.599   1.359  35.684  1.00 33.31
ATOM    668  CB  ASN A  96      10.558  -0.099  37.429  1.00 53.70
ATOM    669  CG  ASN A  96       9.238   0.342  38.026  1.00 61.69
ATOM    670  OD1 ASN A  96       8.758   1.432  37.706  1.00 64.33
ATOM    671  ND2 ASN A  96       8.676  -0.526  38.861  1.00 67.25
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ATOM    672  N   THR A  97      12.287  -0.409  34.507  1.00 30.32
ATOM    673  CA  THR A  97      13.519  -0.367  33.794  1.00 22.83
ATOM    674  C   THR A  97      13.404   0.493  32.534  1.00 22.44
ATOM    675  O   THR A  97      12.446   0.779  31.816  1.00 21.14
ATOM    676  CB  THR A  97      13.835  -1.851  33.705  1.00 25.87
ATOM    677  OG1 THR A  97      14.602  -1.915  32.528  1.00 38.91
ATOM    678  CG2 THR A  97      12.769  -2.901  33.621  1.00 24.22
ATOM    679  N   PRO A  98      14.393   1.415  32.408  1.00 20.59
ATOM    680  CA  PRO A  98      14.513   2.292  31.254  1.00 18.15
ATOM    681  C   PRO A  98      14.882   1.494  29.978  1.00 16.07
ATOM    682  O   PRO A  98      15.622   0.462  29.934  1.00 17.19
ATOM    683  CB  PRO A  98      15.563   3.339  31.676  1.00 14.55
ATOM    684  CG  PRO A  98      16.270   2.646  32.699  1.00 12.29
ATOM    685  CD  PRO A  98      15.735   1.331  33.046  1.00 12.02
ATOM    686  N   VAL A  99      14.322   2.107  28.940  1.00 13.81
ATOM    687  CA  VAL A  99      14.225   1.544  27.632  1.00 14.02
ATOM    688  C   VAL A  99      14.956   2.407  26.663  1.00 10.66
ATOM    689  O   VAL A  99      14.716   3.679  26.712  1.00  6.90
ATOM    690  CB  VAL A  99      12.673   1.343  27.335  1.00  2.87
ATOM    691  CG1 VAL A  99      12.666   1.272  25.872  1.00 17.40
ATOM    692  CG2 VAL A  99      12.442  -0.111  27.744  1.00  5.75
ATOM    693  N   VAL A 100      15.885   1.776  25.861  1.00  6.45
ATOM    694  CA  VAL A 100      16.525   2.755  24.900  1.00  9.61
ATOM    695  C   VAL A 100      16.389   2.159  23.561  1.00 10.79
ATOM    696  O   VAL A 100      16.256   0.973  23.477  1.00  9.11
ATOM    697  CB  VAL A 100      17.877   3.260  25.197  1.00  8.05
ATOM    698  CG1 VAL A 100      17.824   4.252  26.336  1.00  6.05
ATOM    699  CG2 VAL A 100      18.853   2.053  25.591  1.00  6.68
ATOM    700  N   ALA A 101      16.277   2.928  22.511  1.00 13.14
ATOM    701  CA  ALA A 101      16.127   2.266  21.183  1.00 15.67
ATOM    702  C   ALA A 101      17.065   2.747  20.053  1.00 12.08
ATOM    703  O   ALA A 101      17.261   4.042  19.907  1.00 11.16
ATOM    704  CB  ALA A 101      14.685   2.609  20.812  1.00  6.57
ATOM    705  N   GLY A 102      17.218   1.787  19.099  1.00  7.53
ATOM    706  CA  GLY A 102      17.949   2.415  17.939  1.00  7.10
ATOM    707  C   GLY A 102      17.477   1.803  16.744  1.00  7.27
ATOM    708  O   GLY A 102      17.102   0.621  16.878  1.00 10.83
ATOM    709  N   GLY A 103      17.706   2.407  15.648  1.00  7.80
ATOM    710  CA  GLY A 103      17.446   1.745  14.356  1.00  5.33
ATOM    711  C   GLY A 103      18.303   2.211  13.180  1.00  7.56
ATOM    712  O   GLY A 103      18.785   3.340  13.227  1.00  6.88
ATOM    713  N   TYR A 104      18.490   1.387  12.139  1.00  7.09
ATOM    714  CA  TYR A 104      19.392   1.682  11.069  1.00  5.99
ATOM    715  C   TYR A 104      18.705   1.614   9.705  1.00  9.47
ATOM    716  O   TYR A 104      18.115   0.638   9.441  1.00  6.46
ATOM    717  CB  TYR A 104      20.592   0.797  11.079  1.00  5.40
ATOM    718  CG  TYR A 104      21.436   1.078   9.876  1.00  8.05
ATOM    719  CD1 TYR A 104      21.708   2.302   9.352  1.00  5.91
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ATOM    720  CD2 TYR A 104      21.961  -0.044   9.172  1.00  6.85
ATOM    721  CE1 TYR A 104      22.447   2.513   8.186  1.00  5.61
ATOM    722  CE2 TYR A 104      22.751   0.052   8.072  1.00  7.49
ATOM    723  CZ  TYR A 104      22.972   1.377   7.608  1.00 11.08
ATOM    724  OH  TYR A 104      23.795   1.509   6.479  1.00 14.32
ATOM    725  N   SER A 105      18.939   2.975   8.852  1.00 18.39
ATOM    726  CA  SER A 105      18.190   2.854   7.601  1.00  9.66
ATOM    727  C   SER A 105      16.763   2.370   7.722  1.00  6.10
ATOM    728  O   SER A 105      16.090   3.304   8.077  1.00  5.63
ATOM    729  CB  SER A 105      19.124   2.159   6.607  1.00  8.55
ATOM    730  OG  SER A 105      18.553   1.685   5.463  1.00 24.30
ATOM    731  N   GLN A 106      16.241   1.405   7.079  1.00  9.93
ATOM    732  CA  GLN A 106      14.759   1.316   7.002  1.00  8.25
ATOM    733  C   GLN A 106      14.453   1.089   8.473  1.00  8.51
ATOM    734  O   GLN A 106      13.470   1.683   8.862  1.00  6.31
ATOM    735  CB  GLN A 106      14.239   0.393   5.940  1.00  7.45
ATOM    736  CG  GLN A 106      13.184  -0.528   6.465  1.00 18.04
ATOM    737  CD  GLN A 106      12.228  -1.220   5.581  1.00 16.87
ATOM    738  OE1 GLN A 106      11.024  -1.180   5.492  1.00 17.59
ATOM    739  NE2 GLN A 106      12.643  -2.032   4.713  1.00  8.32
ATOM    740  N   GLY A 107      15.269   0.310   9.172  1.00  7.13
ATOM    741  CA  GLY A 107      15.190   0.159  10.606  1.00  4.61
ATOM    742  C   GLY A 107      15.048   1.472  11.356  1.00  8.27
ATOM    743  O   GLY A 107      14.219   1.511  12.290  1.00  6.52
ATOM    744  N   ALA A 108      15.653   2.637  11.033  1.00  6.44
ATOM    745  CA  ALA A 108      15.266   3.864  11.641  1.00  7.41
ATOM    746  C   ALA A 108      13.813   4.346  11.471  1.00 11.76
ATOM    747  O   ALA A 108      13.150   4.914  12.298  1.00 12.64
ATOM    748  CB  ALA A 108      16.121   5.006  11.170  1.00 13.93
ATOM    749  N   ALA A 109      13.321   4.312  10.267  1.00  9.78
ATOM    750  CA  ALA A 109      12.056   4.685   9.861  1.00 10.47
ATOM    751  C   ALA A 109      11.093   3.858  10.727  1.00 12.32
ATOM    752  O   ALA A 109      10.016   4.391  11.035  1.00 14.67
ATOM    753  CB  ALA A 109      12.035   4.173   8.456  1.00 10.24
ATOM    754  N   LEU A 110      11.259   2.690  11.077  1.00  4.34
ATOM    755  CA  LEU A 110      10.458   1.760  11.783  1.00 11.71
ATOM    756  C   LEU A 110      10.305   2.253  13.203  1.00 15.26
ATOM    757  O   LEU A 110       9.298   2.672  13.685  1.00 18.07
ATOM    758  CB  LEU A 110      11.031   0.319  11.634  1.00  7.52
ATOM    759  CG  LEU A 110      10.247  -0.801  12.258  1.00  8.41
ATOM    760  CD1 LEU A 110      10.685  -2.233  11.862  1.00  7.17
ATOM    761  CD2 LEU A 110      10.278  -0.659  13.783  1.00  5.25
ATOM    762  N   ILE A 111      11.397   2.373  13.907  1.00 15.77
ATOM    763  CA  ILE A 111      11.510   2.860  15.246  1.00 12.22
ATOM    764  C   ILE A 111      11.027   4.255  15.234  1.00  9.39
ATOM    765  O   ILE A 111      10.404   4.636  16.241  1.00 12.54
ATOM    766  CB  ILE A 111      12.977   2.814  15.685  1.00 15.55
ATOM    767  CG1 ILE A 111      13.222   1.279  15.805  1.00 14.19
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ATOM    768  CG2 ILE A 111      13.195   3.465  17.005  1.00  4.64
ATOM    769  CD1 ILE A 111      12.410   0.887  17.002  1.00 14.88
ATOM    770  N   ALA A 112      11.309   5.170  14.341  1.00 11.00
ATOM    771  CA  ALA A 112      10.792   6.528  14.427  1.00 12.45
ATOM    772  C   ALA A 112       9.266   6.455  14.308  1.00 15.59
ATOM    773  O   ALA A 112       8.728   7.131  15.154  1.00 18.13
ATOM    774  CB  ALA A 112      11.334   7.505  13.486  1.00  5.70
ATOM    775  N   ALA A 113       8.575   5.572  13.587  1.00 12.85
ATOM    776  CA  ALA A 113       7.167   5.512  13.557  1.00 15.39
ATOM    777  C   ALA A 113       6.475   5.093  14.861  1.00 18.21
ATOM    778  O   ALA A 113       5.498   5.750  15.226  1.00 14.59
ATOM    779  CB  ALA A 113       6.678   4.562  12.500  1.00 17.63
ATOM    780  N   ALA A 114       6.937   3.948  15.303  1.00 16.02
ATOM    781  CA  ALA A 114       6.483   3.218  16.412  1.00 16.43
ATOM    782  C   ALA A 114       6.578   4.114  17.643  1.00 22.20
ATOM    783  O   ALA A 114       5.673   4.321  18.426  1.00 18.94
ATOM    784  CB  ALA A 114       7.474   2.084  16.565  1.00  4.69
ATOM    785  N   VAL A 115       7.722   4.836  17.744  1.00 22.46
ATOM    786  CA  VAL A 115       7.855   5.499  19.064  1.00 20.88
ATOM    787  C   VAL A 115       6.670   6.469  19.007  1.00 22.71
ATOM    788  O   VAL A 115       6.136   6.761  20.057  1.00 22.05
ATOM    789  CB  VAL A 115       9.279   6.090  19.137  1.00 19.61
ATOM    790  CG1 VAL A 115       9.396   7.259  20.122  1.00  8.35
ATOM    791  CG2 VAL A 115      10.245   5.016  19.562  1.00 13.91
ATOM    792  N   SER A 116       6.467   7.085  17.828  1.00 23.59
ATOM    793  CA  SER A 116       5.539   8.172  17.736  1.00 23.68
ATOM    794  C   SER A 116       4.169   7.647  18.120  1.00 23.77
ATOM    795  O   SER A 116       3.333   8.523  18.399  1.00 27.35
ATOM    796  CB  SER A 116       5.522   8.865  16.376  1.00 25.21
ATOM    797  OG  SER A 116       5.168   8.043  15.277  1.00 28.05
ATOM    798  N   GLU A 117       3.859   6.397  18.004  1.00 18.83
ATOM    799  CA  GLU A 117       2.491   6.020  18.238  1.00 22.21
ATOM    800  C   GLU A 117       2.461   5.474  19.653  1.00 30.46
ATOM    801  O   GLU A 117       1.487   4.773  19.863  1.00 35.72
ATOM    802  CB  GLU A 117       1.977   4.902  17.343  1.00 21.63
ATOM    803  CG  GLU A 117       2.167   5.219  15.897  1.00 26.41
ATOM    804  CD  GLU A 117       1.560   4.424  14.814  1.00 34.01
ATOM    805  OE1 GLU A 117       0.912   3.440  15.046  1.00 32.59
ATOM    806  OE2 GLU A 117       1.750   4.833  13.659  1.00 44.62
ATOM    807  N   LEU A 118       3.438   5.570  20.512  1.00 34.45
ATOM    808  CA  LEU A 118       3.326   5.006  21.812  1.00 33.64
ATOM    809  C   LEU A 118       2.681   6.110  22.633  1.00 41.75
ATOM    810  O   LEU A 118       2.594   7.267  22.370  1.00 39.90
ATOM    811  CB  LEU A 118       4.600   4.668  22.392  1.00 29.44
ATOM    812  CG  LEU A 118       5.628   3.891  21.645  1.00 26.36
ATOM    813  CD1 LEU A 118       6.921   3.840  22.379  1.00 27.53
ATOM    814  CD2 LEU A 118       5.110   2.520  21.536  1.00 20.69
ATOM    815  N   SER A 119       2.076   5.794  23.726  1.00 48.86
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ATOM    816  CA  SER A 119       0.910   5.647  24.476  1.00 52.44
ATOM    817  C   SER A 119       1.212   6.063  25.866  1.00 52.57
ATOM    818  O   SER A 119       1.485   5.258  26.735  1.00 55.54
ATOM    819  CB  SER A 119       0.550   4.132  24.488  1.00 70.55
ATOM    820  OG  SER A 119       1.393   3.091  23.908  1.00 66.80
ATOM    821  N   GLY A 120       1.532   7.307  26.024  1.00 52.95
ATOM    822  CA  GLY A 120       1.910   7.761  27.382  1.00 53.35
ATOM    823  C   GLY A 120       2.944   7.109  28.291  1.00 49.09
ATOM    824  O   GLY A 120       4.086   7.617  28.358  1.00 49.66
ATOM    825  N   ALA A 121       2.526   6.129  29.102  1.00 42.97
ATOM    826  CA  ALA A 121       3.477   5.574  30.022  1.00 40.72
ATOM    827  C   ALA A 121       4.587   4.772  29.326  1.00 44.20
ATOM    828  O   ALA A 121       5.749   4.803  29.711  1.00 45.42
ATOM    829  CB  ALA A 121       2.965   4.542  30.903  1.00 36.34
ATOM    830  N   VAL A 122       4.122   4.035  28.312  1.00 41.15
ATOM    831  CA  VAL A 122       5.090   3.269  27.548  1.00 33.41
ATOM    832  C   VAL A 122       5.870   4.168  26.652  1.00 28.48
ATOM    833  O   VAL A 122       7.084   4.019  26.872  1.00 27.69
ATOM    834  CB  VAL A 122       4.424   2.056  26.952  1.00 30.22
ATOM    835  CG1 VAL A 122       2.924   1.997  27.098  1.00 28.03
ATOM    836  CG2 VAL A 122       4.891   1.836  25.551  1.00 23.22
ATOM    837  N   LYS A 123       5.424   5.310  26.177  1.00 23.16
ATOM    838  CA  LYS A 123       6.354   6.314  25.661  1.00 23.11
ATOM    839  C   LYS A 123       7.403   6.783  26.661  1.00 25.28
ATOM    840  O   LYS A 123       8.524   7.224  26.449  1.00 29.01
ATOM    841  CB  LYS A 123       5.561   7.502  25.100  1.00 23.54
ATOM    842  CG  LYS A 123       6.171   8.573  24.277  1.00 26.71
ATOM    843  CD  LYS A 123       5.400   9.775  23.888  1.00 43.07
ATOM    844  CE  LYS A 123       4.953   9.783  22.461  1.00 59.59
ATOM    845  NZ  LYS A 123       3.518   9.637  22.099  1.00 67.50
ATOM    846  N   GLU A 124       6.977   6.991  27.918  1.00 27.95
ATOM    847  CA  GLU A 124       7.845   7.700  28.863  1.00 27.29
ATOM    848  C   GLU A 124       8.910   6.706  29.243  1.00 25.21
ATOM    849  O   GLU A 124       9.993   7.165  29.769  1.00 21.21
ATOM    850  CB  GLU A 124       6.986   8.351  29.927  1.00 40.13
ATOM    851  CG  GLU A 124       7.588   8.609  31.295  1.00 57.40
ATOM    852  CD  GLU A 124       8.530   9.814  31.247  1.00 66.99
ATOM    853  OE1 GLU A 124       9.619   9.751  31.902  1.00 70.44
ATOM    854  OE2 GLU A 124       7.949  10.652  30.502  1.00 73.84
ATOM    855  N   GLN A 125       8.656   5.393  29.058  1.00 19.93
ATOM    856  CA  GLN A 125       9.761   4.509  29.546  1.00 17.98
ATOM    857  C   GLN A 125      10.865   4.556  28.521  1.00 24.28
ATOM    858  O   GLN A 125      11.964   4.107  28.815  1.00 21.47
ATOM    859  CB  GLN A 125       9.225   3.178  29.844  1.00  9.13
ATOM    860  CG  GLN A 125       9.901   2.001  30.299  1.00  9.05
ATOM    861  CD  GLN A 125       9.211   0.719  30.129  1.00 19.33
ATOM    862  OE1 GLN A 125       8.190   0.703  29.466  1.00 28.52
ATOM    863  NE2 GLN A 125       9.662  -0.396  30.684  1.00 13.34
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ATOM    864  N   VAL A 126      10.593   5.188  27.319  1.00 25.30
ATOM    865  CA  VAL A 126      11.738   5.124  26.361  1.00 22.55
ATOM    866  C   VAL A 126      12.546   6.334  26.614  1.00 17.55
ATOM    867  O   VAL A 126      12.109   7.408  26.329  1.00 12.79
ATOM    868  CB  VAL A 126      11.227   4.560  25.022  1.00 23.76
ATOM    869  CG1 VAL A 126       9.706   4.686  24.946  1.00 23.77
ATOM    870  CG2 VAL A 126      11.795   5.081  23.743  1.00 23.81
ATOM    871  N   LYS A 127      13.726   6.233  27.264  1.00 16.41
ATOM    872  CA  LYS A 127      14.462   7.494  27.639  1.00 18.18
ATOM    873  C   LYS A 127      15.239   8.063  26.488  1.00 18.49
ATOM    874  O   LYS A 127      15.812   9.103  26.680  1.00 18.99
ATOM    875  CB  LYS A 127      15.401   7.148  28.792  1.00 20.81
ATOM    876  CG  LYS A 127      14.770   6.110  29.713  1.00 21.99
ATOM    877  CD  LYS A 127      13.435   6.726  30.064  1.00 33.86
ATOM    878  CE  LYS A 127      12.779   6.612  31.399  1.00 32.17
ATOM    879  NZ  LYS A 127      12.279   7.863  31.993  1.00 45.34
ATOM    880  N   GLY A 128      15.522   7.281  25.416  1.00 20.56
ATOM    881  CA  GLY A 128      16.280   7.948  24.306  1.00 20.72
ATOM    882  C   GLY A 128      16.358   7.104  23.063  1.00 17.71
ATOM    883  O   GLY A 128      16.168   5.901  23.226  1.00 16.66
ATOM    884  N   VAL A 129      16.451   7.725  21.892  1.00 16.16
ATOM    885  CA  VAL A 129      16.497   6.872  20.691  1.00 13.82
ATOM    886  C   VAL A 129      17.519   7.371  19.719  1.00  8.35
ATOM    887  O   VAL A 129      17.602   8.553  19.556  1.00  3.85
ATOM    888  CB  VAL A 129      15.192   6.426  20.054  1.00 11.02
ATOM    889  CG1 VAL A 129      14.007   7.041  20.726  1.00  6.50
ATOM    890  CG2 VAL A 129      15.051   6.729  18.571  1.00 10.03
ATOM    891  N   ALA A 130      18.455   6.398  19.363  1.00  8.05
ATOM    892  CA  ALA A 130      19.430   6.845  18.344  1.00  7.55
ATOM    893  C   ALA A 130      19.078   6.293  16.958  1.00 11.17
ATOM    894  O   ALA A 130      18.755   5.145  16.849  1.00 15.74
ATOM    895  CB  ALA A 130      20.781   6.391  18.603  1.00  5.89
ATOM    896  N   LEU A 131      18.911   6.953  15.892  1.00  7.36
ATOM    897  CA  LEU A 131      18.635   6.625  14.553  1.00  7.70
ATOM    898  C   LEU A 131      19.876   6.908  13.661  1.00 12.02
ATOM    899  O   LEU A 131      20.436   8.033  13.604  1.00  6.80
ATOM    900  CB  LEU A 131      17.604   7.713  14.102  1.00  8.40
ATOM    901  CG  LEU A 131      16.160   7.830  14.575  1.00  6.67
ATOM    902  CD1 LEU A 131      15.391   8.957  13.981  1.00  4.49
ATOM    903  CD2 LEU A 131      15.481   6.488  14.324  1.00  5.12
ATOM    904  N   PHE A 132      20.271   6.009  12.802  1.00 11.56
ATOM    905  CA  PHE A 132      21.422   6.183  11.908  1.00 10.44
ATOM    906  C   PHE A 132      20.965   6.013  10.478  1.00  8.46
ATOM    907  O   PHE A 132      20.175   5.101  10.097  1.00 11.04
ATOM    908  CB  PHE A 132      22.217   4.931  12.282  1.00 10.56
ATOM    909  CG  PHE A 132      22.693   4.830  13.714  1.00 16.38
ATOM    910  CD1 PHE A 132      21.951   4.029  14.542  1.00 13.36
ATOM    911  CD2 PHE A 132      23.860   5.489  14.213  1.00 15.12
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ATOM    912  CE1 PHE A 132      22.342   3.911  15.889  1.00 14.91
ATOM    913  CE2 PHE A 132      24.176   5.323  15.513  1.00 18.02
ATOM    914  CZ  PHE A 132      23.426   4.530  16.403  1.00 15.09
ATOM    915  N   GLY A 133      21.431   6.876   9.580  1.00  7.35
ATOM    916  CA  GLY A 133      21.026   6.893   8.148  1.00  5.86
ATOM    917  C   GLY A 133      19.503   6.919   8.061  1.00 12.25
ATOM    918  O   GLY A 133      18.890   5.926   7.593  1.00  9.03
ATOM    919  N   TYR A 134      18.926   8.070   8.532  1.00  9.85
ATOM    920  CA  TYR A 134      17.455   8.022   8.838  1.00  7.40
ATOM    921  C   TYR A 134      16.647   8.365   7.584  1.00 10.61
ATOM    922  O   TYR A 134      16.785   9.513   7.131  1.00  5.85
ATOM    923  CB  TYR A 134      17.161   9.128   9.836  1.00  7.27
ATOM    924  CG  TYR A 134      15.842   9.393  10.391  1.00  7.89
ATOM    925  CD1 TYR A 134      14.889   8.437  10.312  1.00  6.65
ATOM    926  CD2 TYR A 134      15.661  10.651  10.948  1.00 11.44
ATOM    927  CE1 TYR A 134      13.657   8.690  10.821  1.00  9.05
ATOM    928  CE2 TYR A 134      14.408  10.928  11.467  1.00 12.89
ATOM    929  CZ  TYR A 134      13.428   9.923  11.423  1.00 14.22
ATOM    930  OH  TYR A 134      12.146  10.110  11.975  1.00 12.41
ATOM    931  N   THR A 135      15.811   7.398   7.139  1.00 11.51
ATOM    932  CA  THR A 135      15.229   7.581   5.789  1.00  7.71
ATOM    933  C   THR A 135      14.082   8.530   5.825  1.00 10.36
ATOM    934  O   THR A 135      13.845   8.878   4.727  1.00 11.26
ATOM    935  CB  THR A 135      14.772   6.394   4.967  1.00 12.02
ATOM    936  OG1 THR A 135      13.821   5.399   5.398  1.00 22.81
ATOM    937  CG2 THR A 135      15.828   5.332   4.712  1.00 14.88
ATOM    938  N   GLN A 136      13.632   9.105   6.928  1.00 15.28
ATOM    939  CA  GLN A 136      12.596  10.134   6.968  1.00 16.48
ATOM    940  C   GLN A 136      13.102  11.418   7.646  1.00 17.46
ATOM    941  O   GLN A 136      12.292  12.231   8.035  1.00 12.82
ATOM    942  CB  GLN A 136      11.336   9.671   7.701  1.00  5.71
ATOM    943  CG  GLN A 136      11.178   8.191   7.263  1.00 13.60
ATOM    944  CD  GLN A 136      10.504   8.264   5.932  1.00 14.65
ATOM    945  OE1 GLN A 136       9.587   9.102   5.986  1.00 23.99
ATOM    946  NE2 GLN A 136      10.852   7.529   4.914  1.00 14.68
ATOM    947  N   ASN A 137      14.421  11.532   7.566  1.00 18.52
ATOM    948  CA  ASN A 137      14.953  12.752   8.141  1.00 18.16
ATOM    949  C   ASN A 137      14.301  13.929   7.458  1.00 19.79
ATOM    950  O   ASN A 137      13.895  14.802   8.157  1.00 12.28
ATOM    951  CB  ASN A 137      16.481  12.573   8.239  1.00 14.17
ATOM    952  CG  ASN A 137      17.247  13.740   8.812  1.00 19.75
ATOM    953  OD1 ASN A 137      17.821  14.341   7.934  1.00 14.52
ATOM    954  ND2 ASN A 137      17.390  14.130  10.042  1.00 17.43
ATOM    955  N   LEU A 138      14.180  14.062   6.141  1.00 27.31
ATOM    956  CA  LEU A 138      13.640  15.270   5.553  1.00 25.53
ATOM    957  C   LEU A 138      12.190  15.332   5.971  1.00 22.45
ATOM    958  O   LEU A 138      11.710  16.281   6.549  1.00 25.13
ATOM    959  CB  LEU A 138      13.632  15.269   4.056  1.00 41.28
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ATOM    960  CG  LEU A 138      13.713  16.582   3.303  1.00 31.76
ATOM    961  CD1 LEU A 138      14.641  17.503   4.012  1.00 51.09
ATOM    962  CD2 LEU A 138      14.207  16.573   1.958  1.00 46.20
ATOM    963  N   GLN A 139      11.378  14.403   5.569  1.00 20.48
ATOM    964  CA  GLN A 139      10.034  14.390   6.037  1.00 19.98
ATOM    965  C   GLN A 139       9.846  14.749   7.471  1.00 22.85
ATOM    966  O   GLN A 139       8.791  15.282   7.528  1.00 26.66
ATOM    967  CB  GLN A 139       9.517  12.969   5.899  1.00 18.37
ATOM    968  CG  GLN A 139       9.684  12.643   4.450  1.00 22.02
ATOM    969  CD  GLN A 139      10.984  11.983   4.110  1.00 22.69
ATOM    970  OE1 GLN A 139      10.674  10.980   3.477  1.00 35.62
ATOM    971  NE2 GLN A 139      12.195  12.405   4.410  1.00 31.70
ATOM    972  N   ASN A 140      10.454  14.072   8.427  1.00 26.14
ATOM    973  CA  ASN A 140      10.215  14.183   9.848  1.00 19.06
ATOM    974  C   ASN A 140      10.941  15.429  10.293  1.00 16.99
ATOM    975  O   ASN A 140      11.040  15.654  11.454  1.00 18.05
ATOM    976  CB  ASN A 140      10.581  12.910  10.541  1.00 17.20
ATOM    977  CG  ASN A 140       9.465  11.998  10.210  1.00 16.28
ATOM    978  OD1 ASN A 140       8.615  12.565   9.563  1.00 23.57
ATOM    979  ND2 ASN A 140       9.460  10.756  10.630  1.00 22.65
ATOM    980  N   ARG A 141      11.457  16.162   9.397  1.00 19.20
ATOM    981  CA  ARG A 141      12.170  17.350   9.790  1.00 26.25
ATOM    982  C   ARG A 141      13.219  17.090  10.818  1.00 25.06
ATOM    983  O   ARG A 141      13.365  17.928  11.649  1.00 27.60
ATOM    984  CB  ARG A 141      11.123  18.299  10.271  1.00 37.72
ATOM    985  CG  ARG A 141      10.083  18.974   9.372  1.00 49.61
ATOM    986  N   GLY A 142      14.110  16.165  10.920  1.00 19.42
ATOM    987  CA  GLY A 142      14.997  15.778  11.902  1.00 14.21
ATOM    988  C   GLY A 142      14.652  15.066  13.158  1.00 19.42
ATOM    989  O   GLY A 142      15.547  14.759  13.971  1.00 23.74
ATOM    990  N   GLY A 143      13.354  14.851  13.569  1.00 14.09
ATOM    991  CA  GLY A 143      13.210  14.075  14.757  1.00 11.80
ATOM    992  C   GLY A 143      12.203  12.972  14.555  1.00 16.69
ATOM    993  O   GLY A 143      11.760  12.787  13.481  1.00 19.57
ATOM    994  N   ILE A 144      11.668  12.386  15.590  1.00 19.71
ATOM    995  CA  ILE A 144      10.494  11.589  15.667  1.00 20.13
ATOM    996  C   ILE A 144       9.313  12.315  16.296  1.00 27.00
ATOM    997  O   ILE A 144       9.298  13.026  17.268  1.00 26.75
ATOM    998  CB  ILE A 144      10.973  10.583  16.692  1.00 16.84
ATOM    999  CG1 ILE A 144      12.363   9.956  16.348  1.00  5.60
ATOM   1000  CG2 ILE A 144       9.882   9.636  16.775  1.00 14.01
ATOM   1001  CD1 ILE A 144      12.437   9.156  17.562  1.00  2.75
ATOM   1002  N   PRO A 145       8.249  12.380  15.499  1.00 32.77
ATOM   1003  CA  PRO A 145       6.959  12.993  15.779  1.00 29.89
ATOM   1004  C   PRO A 145       6.484  12.588  17.180  1.00 27.78
ATOM   1005  O   PRO A 145       6.475  11.446  17.537  1.00 26.07
ATOM   1006  CB  PRO A 145       5.957  12.384  14.784  1.00 26.51
ATOM   1007  CG  PRO A 145       6.887  12.059  13.668  1.00 25.85
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ATOM   1008  CD  PRO A 145       8.174  11.563  14.234  1.00 31.33
ATOM   1009  N   ASN A 146       5.796  13.462  17.878  1.00 27.07
ATOM   1010  CA  ASN A 146       5.454  13.274  19.230  1.00 28.59
ATOM   1011  C   ASN A 146       6.526  12.605  20.045  1.00 29.25
ATOM   1012  O   ASN A 146       6.087  11.995  20.996  1.00 35.51
ATOM   1013  CB  ASN A 146       4.285  12.364  19.230  1.00 41.13
ATOM   1014  CG  ASN A 146       3.300  12.568  18.120  1.00 48.43
ATOM   1015  OD1 ASN A 146       3.134  13.721  17.788  1.00 49.24
ATOM   1016  ND2 ASN A 146       2.763  11.437  17.695  1.00 47.79
ATOM   1017  N   TYR A 147       7.791  12.799  19.885  1.00 23.88
ATOM   1018  CA  TYR A 147       8.689  12.339  20.969  1.00 21.90
ATOM   1019  C   TYR A 147       9.583  13.495  21.285  1.00 22.57
ATOM   1020  O   TYR A 147       9.777  14.399  20.494  1.00 26.53
ATOM   1021  CB  TYR A 147       9.309  11.098  20.498  1.00 21.16
ATOM   1022  CG  TYR A 147      10.285  10.471  21.349  1.00 20.45
ATOM   1023  CD1 TYR A 147       9.882   9.720  22.384  1.00 24.28
ATOM   1024  CD2 TYR A 147      11.608  10.564  21.189  1.00 17.96
ATOM   1025  CE1 TYR A 147      10.681   9.029  23.273  1.00 24.55
ATOM   1026  CE2 TYR A 147      12.509   9.948  21.983  1.00 20.73
ATOM   1027  CZ  TYR A 147      12.022   9.184  23.030  1.00 24.61
ATOM   1028  OH  TYR A 147      12.891   8.536  23.887  1.00 24.80
ATOM   1029  N   PRO A 148       9.893  13.858  22.507  1.00 22.86
ATOM   1030  CA  PRO A 148      10.817  14.916  22.769  1.00 21.77
ATOM   1031  C   PRO A 148      12.127  14.882  21.957  1.00 22.49
ATOM   1032  O   PRO A 148      13.007  14.004  22.117  1.00 22.31
ATOM   1033  CB  PRO A 148      11.185  14.694  24.251  1.00 23.23
ATOM   1034  CG  PRO A 148      10.324  13.576  24.719  1.00 23.39
ATOM   1035  CD  PRO A 148       9.677  12.889  23.590  1.00 25.33
ATOM   1036  N   ARG A 149      12.432  15.980  21.250  1.00 25.45
ATOM   1037  CA  ARG A 149      13.735  16.138  20.567  1.00 22.54
ATOM   1038  C   ARG A 149      14.910  16.018  21.499  1.00 21.28
ATOM   1039  O   ARG A 149      15.860  15.477  21.015  1.00 16.61
ATOM   1040  CB  ARG A 149      13.829  17.346  19.727  1.00 31.02
ATOM   1041  CG  ARG A 149      12.837  17.750  18.719  1.00 58.26
ATOM   1042  CD  ARG A 149      13.452  18.605  17.658  1.00 80.58
ATOM   1043  NE  ARG A 149      13.769  17.798  16.491  1.00 92.05
ATOM   1044  CZ  ARG A 149      13.315  18.154  15.320  1.00 91.85
ATOM   1045  NH1 ARG A 149      12.586  19.213  15.165  1.00 86.98
ATOM   1046  NH2 ARG A 149      13.544  17.488  14.242  1.00 91.61
ATOM   1047  N   GLU A 150      14.813  16.282  22.825  1.00 28.09
ATOM   1048  CA  GLU A 150      15.950  16.171  23.735  1.00 25.55
ATOM   1049  C   GLU A 150      16.272  14.736  24.020  1.00 21.12
ATOM   1050  O   GLU A 150      17.372  14.443  24.371  1.00 24.39
ATOM   1051  CB  GLU A 150      15.753  17.040  24.917  1.00 38.73
ATOM   1052  CG  GLU A 150      14.328  17.370  25.359  1.00 67.27
ATOM   1053  CD  GLU A 150      14.252  17.185  26.899  1.00 85.05
ATOM   1054  OE1 GLU A 150      15.005  17.890  27.657  1.00 90.70
ATOM   1055  OE2 GLU A 150      13.454  16.321  27.373  1.00 91.68
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ATOM   1056  N   ARG A 151      15.396  13.807  23.727  1.00 19.70
ATOM   1057  CA  ARG A 151      15.752  12.424  23.844  1.00 19.52
ATOM   1058  C   ARG A 151      16.163  11.779  22.531  1.00 19.28
ATOM   1059  O   ARG A 151      16.373  10.586  22.480  1.00 14.55
ATOM   1060  CB  ARG A 151      14.548  11.796  24.412  1.00 23.06
ATOM   1061  CG  ARG A 151      13.853  12.432  25.516  1.00 22.24
ATOM   1062  CD  ARG A 151      13.200  11.451  26.393  1.00 33.40
ATOM   1063  NE  ARG A 151      12.609  11.893  27.633  1.00 46.53
ATOM   1064  CZ  ARG A 151      11.796  11.028  28.275  1.00 52.87
ATOM   1065  NH1 ARG A 151      11.428   9.823  27.930  1.00 51.02
ATOM   1066  NH2 ARG A 151      11.203  11.278  29.416  1.00 59.98
ATOM   1067  N   THR A 152      16.360  12.526  21.505  1.00 14.12
ATOM   1068  CA  THR A 152      16.629  11.925  20.253  1.00 15.05
ATOM   1069  C   THR A 152      17.995  12.249  19.745  1.00 17.30
ATOM   1070  O   THR A 152      18.282  13.373  19.965  1.00 21.34
ATOM   1071  CB  THR A 152      15.680  12.408  19.158  1.00 13.91
ATOM   1072  OG1 THR A 152      14.423  12.256  19.858  1.00 23.92
ATOM   1073  CG2 THR A 152      15.737  11.934  17.759  1.00  6.77
ATOM   1074  N   LYS A 153      18.704  11.336  19.121  1.00 15.49
ATOM   1075  CA  LYS A 153      19.930  11.725  18.450  1.00 17.73
ATOM   1076  C   LYS A 153      19.893  11.035  17.073  1.00 18.41
ATOM   1077  O   LYS A 153      19.866   9.800  17.121  1.00 16.04
ATOM   1078  CB  LYS A 153      21.112  11.260  19.338  1.00 14.55
ATOM   1079  CG  LYS A 153      22.523  11.508  18.933  1.00 11.95
ATOM   1080  CD  LYS A 153      22.883  12.882  19.403  1.00 40.35
ATOM   1081  CE  LYS A 153      24.358  13.093  19.079  1.00 62.12
ATOM   1082  NZ  LYS A 153      24.930  14.235  19.863  1.00 73.03
ATOM   1083  N   VAL A 154      19.910  11.962  16.136  1.00 15.86
ATOM   1084  CA  VAL A 154      20.031  11.508  14.730  1.00 15.79
ATOM   1085  C   VAL A 154      21.406  11.481  14.040  1.00 13.11
ATOM   1086  O   VAL A 154      21.958  12.460  13.675  1.00 13.51
ATOM   1087  CB  VAL A 154      19.095  12.257  13.674  1.00  5.90
ATOM   1088  CG1 VAL A 154      19.276  11.765  12.247  1.00  8.45
ATOM   1089  CG2 VAL A 154      17.672  12.091  14.117  1.00  7.14
ATOM   1090  N   PHE A 155      22.039  10.448  13.605  1.00 13.75
ATOM   1091  CA  PHE A 155      23.263  10.473  12.843  1.00 10.67
ATOM   1092  C   PHE A 155      22.906  10.406  11.402  1.00 11.64
ATOM   1093  O   PHE A 155      22.505   9.367  10.893  1.00 15.09
ATOM   1094  CB  PHE A 155      23.955   9.120  13.304  1.00  5.38
ATOM   1095  CG  PHE A 155      24.396   9.266  14.739  1.00 16.52
ATOM   1096  CD1 PHE A 155      23.678   8.642  15.696  1.00 23.70
ATOM   1097  CD2 PHE A 155      25.503   9.950  15.107  1.00 11.27
ATOM   1098  CE1 PHE A 155      24.037   8.702  17.011  1.00 23.25
ATOM   1099  CE2 PHE A 155      25.888   9.994  16.372  1.00  7.37
ATOM   1100  CZ  PHE A 155      25.139   9.384  17.357  1.00 16.13
ATOM   1101  N   CYS A 156      23.205  11.255  10.511  1.00 12.38
ATOM   1102  CA  CYS A 156      22.847  11.443   9.114  1.00 11.64
ATOM   1103  C   CYS A 156      24.057  12.027   8.461  1.00 10.08
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ATOM	1104	O	CYS	A	156	24.385	13.174	8.378	1.00 13.73
ATOM	1105	CB	CYS	A	156	21.575	12.391	8.917	1.00 6.30
ATOM	1106	SG	CYS	A	156	20.137	11.470	8.287	1.00 10.60
ATOM	1107	N	ASN	A	157	24.814	11.147	7.918	1.00 16.95
ATOM	1108	CA	ASN	A	157	26.229	11.665	7.576	1.00 19.16
ATOM	1109	C	ASN	A	157	26.197	12.367	6.310	1.00 17.70
ATOM	1110	O	ASN	A	157	25.368	12.330	5.469	1.00 20.91
ATOM	1111	CB	ASN	A	157	27.115	10.714	8.300	1.00 30.34
ATOM	1112	CG	ASN	A	157	27.733	9.498	7.932	1.00 34.95
ATOM	1113	OD1	ASN	A	157	28.011	8.573	8.606	1.00 44.28
ATOM	1114	ND2	ASN	A	157	27.965	9.541	6.660	1.00 54.18
ATOM	1115	N	VAL	A	158	26.849	13.501	6.313	1.00 25.65
ATOM	1116	CA	VAL	A	158	26.825	14.483	5.192	1.00 28.21
ATOM	1117	C	VAL	A	158	26.768	13.893	3.758	1.00 24.85
ATOM	1118	O	VAL	A	158	25.732	14.266	3.111	1.00 30.96
ATOM	1119	CB	VAL	A	158	27.954	15.512	5.217	1.00 27.87
ATOM	1120	CG1	VAL	A	158	28.751	14.595	4.238	1.00 40.51
ATOM	1121	CG2	VAL	A	158	27.791	16.704	4.399	1.00 34.39
ATOM	1122	N	GLY	A	159	27.483	12.956	3.016	1.00 5.94
ATOM	1123	CA	GLY	A	159	26.713	12.774	1.732	1.00 6.20
ATOM	1124	C	GLY	A	159	25.734	11.797	1.487	1.00 4.00
ATOM	1125	O	GLY	A	159	25.732	10.704	0.848	1.00 4.06
ATOM	1126	N	ASP	A	160	25.052	11.441	2.643	1.00 8.53
ATOM	1127	CA	ASP	A	160	24.106	10.302	2.828	1.00 11.97
ATOM	1128	C	ASP	A	160	22.755	10.698	2.177	1.00 14.44
ATOM	1129	O	ASP	A	160	21.928	11.398	2.692	1.00 10.21
ATOM	1130	CB	ASP	A	160	24.037	9.829	4.277	1.00 12.43
ATOM	1131	CG	ASP	A	160	23.126	8.629	4.261	1.00 20.99
ATOM	1132	OD1	ASP	A	160	22.525	8.408	3.179	1.00 33.03
ATOM	1133	OD2	ASP	A	160	22.956	7.840	5.216	1.00 10.13
ATOM	1134	N	ALA	A	161	22.455	10.402	0.961	1.00 12.33
ATOM	1135	CA	ALA	A	161	21.318	10.743	0.269	1.00 11.01
ATOM	1136	C	ALA	A	161	19.961	10.317	0.848	1.00 15.22
ATOM	1137	O	ALA	A	161	18.969	11.034	0.594	1.00 9.50
ATOM	1138	CB	ALA	A	161	21.365	10.334	-1.172	1.00 13.68
ATOM	1139	N	VAL	A	162	19.915	9.468	1.840	1.00 14.54
ATOM	1140	CA	VAL	A	162	18.653	9.014	2.287	1.00 9.86
ATOM	1141	C	VAL	A	162	18.235	10.063	3.258	1.00 13.50
ATOM	1142	O	VAL	A	162	17.094	10.458	3.377	1.00 20.47
ATOM	1143	CB	VAL	A	162	18.596	7.778	3.117	1.00 7.34
ATOM	1144	CG1	VAL	A	162	18.931	6.592	2.259	1.00 6.50
ATOM	1145	CG2	VAL	A	162	19.514	7.858	4.210	1.00 18.46
ATOM	1146	N	CYS	A	163	19.198	10.733	3.719	1.00 13.44
ATOM	1147	CA	CYS	A	163	18.864	11.811	4.720	1.00 11.26
ATOM	1148	C	CYS	A	163	18.256	12.963	4.042	1.00 15.57
ATOM	1149	O	CYS	A	163	18.219	13.857	4.880	1.00 14.09
ATOM	1150	CB	CYS	A	163	20.144	12.145	5.570	1.00 18.70
ATOM	1151	SG	CYS	A	163	20.748	10.705	6.581	1.00 13.38

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ATOM   1152  N   THR A 164      18.100  13.014   2.696  1.00 21.82
ATOM   1153  CA  THR A 164      17.603  14.283   2.171  1.00 23.08
ATOM   1154  C   THR A 164      16.597  14.022   1.098  1.00 23.39
ATOM   1155  O   THR A 164      16.517  14.727   0.137  1.00 33.37
ATOM   1156  CB  THR A 164      18.463  15.341   1.454  1.00 23.25
ATOM   1157  OG1 THR A 164      19.486  14.707   0.674  1.00 23.21
ATOM   1158  CG2 THR A 164      18.958  16.261   2.491  1.00 37.71
ATOM   1159  N   GLY A 165      15.802  13.085   1.309  1.00 24.23
ATOM   1160  CA  GLY A 165      14.606  12.783   0.579  1.00 26.69
ATOM   1161  C   GLY A 165      14.699  11.814  -0.515  1.00 28.56
ATOM   1162  O   GLY A 165      13.680  11.775  -1.124  1.00 39.76
ATOM   1163  N   THR A 166      15.661  11.044  -0.736  1.00 25.80
ATOM   1164  CA  THR A 166      16.006  10.220  -1.774  1.00 25.53
ATOM   1165  C   THR A 166      16.195   8.866  -1.175  1.00 25.35
ATOM   1166  O   THR A 166      16.913   8.760  -0.206  1.00 30.91
ATOM   1167  CB  THR A 166      17.406  10.657  -2.230  1.00 31.57
ATOM   1168  OG1 THR A 166      17.105  11.788  -2.982  1.00 24.13
ATOM   1169  CG2 THR A 166      18.061   9.559  -2.983  1.00 34.67
ATOM   1170  N   LEU A 167      15.734   7.833  -1.817  1.00 19.63
ATOM   1171  CA  LEU A 167      16.219   6.552  -1.465  1.00 16.11
ATOM   1172  C   LEU A 167      17.395   6.044  -2.300  1.00 19.87
ATOM   1173  O   LEU A 167      17.265   4.869  -2.612  1.00 21.38
ATOM   1174  CB  LEU A 167      15.086   5.624  -1.555  1.00 23.45
ATOM   1175  CG  LEU A 167      14.123   5.773  -0.401  1.00 33.91
ATOM   1176  CD1 LEU A 167      12.969   4.908  -0.793  1.00 42.10
ATOM   1177  CD2 LEU A 167      14.776   5.385   0.903  1.00 25.86
ATOM   1178  N   ILE A 168      18.534   6.726  -2.507  1.00 21.67
ATOM   1179  CA  ILE A 168      19.608   6.051  -3.170  1.00 23.38
ATOM   1180  C   ILE A 168      20.675   5.585  -2.189  1.00 20.47
ATOM   1181  O   ILE A 168      21.139   6.541  -1.581  1.00 18.08
ATOM   1182  CB  ILE A 168      20.254   6.835  -4.297  1.00 23.50
ATOM   1183  CG1 ILE A 168      21.232   7.874  -3.800  1.00 13.71
ATOM   1184  CG2 ILE A 168      19.445   7.627  -5.276  1.00 18.16
ATOM   1185  CD1 ILE A 168      20.908   8.938  -4.804  1.00 26.95
ATOM   1186  N   ILE A 169      21.396   4.478  -2.394  1.00 18.32
ATOM   1187  CA  ILE A 169      22.554   4.448  -1.536  1.00 13.25
ATOM   1188  C   ILE A 169      23.924   4.662  -1.967  1.00 11.95
ATOM   1189  O   ILE A 169      24.615   3.942  -2.539  1.00 20.35
ATOM   1190  CB  ILE A 169      22.503   3.351  -0.499  1.00 21.07
ATOM   1191  CG1 ILE A 169      23.398   2.181  -0.655  1.00 11.06
ATOM   1192  CG2 ILE A 169      21.122   2.801  -0.533  1.00  7.02
ATOM   1193  CD1 ILE A 169      22.581   1.266  -1.587  1.00 32.83
ATOM   1194  N   THR A 170      24.570   5.586  -1.296  1.00 17.16
ATOM   1195  CA  THR A 170      25.883   6.217  -1.397  1.00 13.01
ATOM   1196  C   THR A 170      26.722   5.719  -0.240  1.00 10.14
ATOM   1197  O   THR A 170      26.334   5.036   0.758  1.00  9.98
ATOM   1198  CB  THR A 170      25.623   7.713  -1.344  1.00 15.02
ATOM   1199  OG1 THR A 170      26.466   7.947  -0.255  1.00 23.39
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ATOM   1200  CG2 THR A 170      24.389   7.914  -0.452  1.00 41.10
ATOM   1201  N   PRO A 171      28.000   5.738  -0.469  1.00 10.12
ATOM   1202  CA  PRO A 171      29.012   5.066   0.339  1.00 11.88
ATOM   1203  C   PRO A 171      28.897   5.492   1.765  1.00  9.74
ATOM   1204  O   PRO A 171      28.904   4.682   2.646  1.00  9.54
ATOM   1205  CB  PRO A 171      30.414   5.207  -0.286  1.00  7.15
ATOM   1206  CG  PRO A 171      30.017   5.603  -1.654  1.00  7.18
ATOM   1207  CD  PRO A 171      28.667   6.233  -1.601  1.00  6.90
ATOM   1208  N   ALA A 172      28.725   6.718   1.980  1.00  6.71
ATOM   1209  CA  ALA A 172      28.247   7.315   3.169  1.00  8.62
ATOM   1210  C   ALA A 172      27.075   6.631   3.892  1.00 10.99
ATOM   1211  O   ALA A 172      27.037   6.755   5.165  1.00 16.49
ATOM   1212  CB  ALA A 172      27.904   8.812   3.040  1.00  2.86
ATOM   1213  N   HIS A 173      26.287   5.815   3.278  1.00  6.36
ATOM   1214  CA  HIS A 173      25.133   5.468   4.081  1.00  5.29
ATOM   1215  C   HIS A 173      25.685   4.314   4.888  1.00 10.58
ATOM   1216  O   HIS A 173      25.082   3.598   5.668  1.00  9.36
ATOM   1217  CB  HIS A 173      24.081   4.883   3.216  1.00  8.41
ATOM   1218  CG  HIS A 173      22.815   4.403   3.791  1.00  7.30
ATOM   1219  ND1 HIS A 173      22.066   5.327   4.565  1.00  8.48
ATOM   1220  CD2 HIS A 173      22.148   3.264   3.670  1.00  7.83
ATOM   1221  CE1 HIS A 173      20.932   4.657   4.861  1.00 17.36
ATOM   1222  NE2 HIS A 173      20.945   3.423   4.379  1.00  5.29
ATOM   1223  N   LEU A 174      26.823   3.947   4.326  1.00  8.03
ATOM   1224  CA  LEU A 174      27.344   2.623   4.682  1.00  8.06
ATOM   1225  C   LEU A 174      28.171   2.787   5.930  1.00 13.06
ATOM   1226  O   LEU A 174      28.609   1.648   6.151  1.00 19.88
ATOM   1227  CB  LEU A 174      28.078   2.118   3.488  1.00  2.76
ATOM   1228  CG  LEU A 174      27.560   0.902   2.847  1.00 13.35
ATOM   1229  CD1 LEU A 174      26.024   1.017   2.796  1.00 18.01
ATOM   1230  CD2 LEU A 174      27.913   0.740   1.421  1.00 21.70
ATOM   1231  N   SER A 175      28.290   3.989   6.447  1.00 12.43
ATOM   1232  CA  SER A 175      29.230   4.052   7.553  1.00 18.01
ATOM   1233  C   SER A 175      28.872   4.811   8.847  1.00 19.89
ATOM   1234  O   SER A 175      28.968   6.047   9.120  1.00 14.61
ATOM   1235  CB  SER A 175      30.516   4.606   6.847  1.00 20.11
ATOM   1236  OG  SER A 175      30.834   5.907   7.293  1.00 27.73
ATOM   1237  N   TYR A 176      28.479   3.978   9.815  1.00 17.89
ATOM   1238  CA  TYR A 176      28.092   4.530  11.133  1.00 12.54
ATOM   1239  C   TYR A 176      28.530   3.671  12.272  1.00 11.16
ATOM   1240  O   TYR A 176      27.949   3.770  13.257  1.00  7.63
ATOM   1241  CB  TYR A 176      26.511   4.283  11.053  1.00  9.13
ATOM   1242  CG  TYR A 176      25.831   5.525  10.029  1.00  5.03
ATOM   1243  CD1 TYR A 176      25.874   6.923  10.425  1.00  2.75
ATOM   1244  CD2 TYR A 176      25.152   5.022   8.980  1.00  2.18
ATOM   1245  CE1 TYR A 176      25.287   7.754   9.633  1.00  4.25
ATOM   1246  CE2 TYR A 176      24.649   5.981   8.085  1.00  6.77
ATOM   1247  CZ  TYR A 176      24.658   7.329   8.399  1.00  6.22
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ATOM   1248  OH  TYR A 176      24.074   8.375   7.635  1.00  5.76
ATOM   1249  N   THR A 177      29.430   2.685  12.167  1.00 10.72
ATOM   1250  CA  THR A 177      29.797   1.854  13.284  1.00 13.31
ATOM   1251  C   THR A 177      30.516   2.659  14.320  1.00 12.46
ATOM   1252  O   THR A 177      30.311   2.436  15.475  1.00 13.12
ATOM   1253  CB  THR A 177      30.658   0.683  12.798  1.00  3.49
ATOM   1254  OG1 THR A 177      31.361   1.247  11.870  1.00 32.08
ATOM   1255  CG2 THR A 177      29.675  -0.149  12.083  1.00  6.42
ATOM   1256  N   ILE A 178      31.409   3.474  13.920  1.00 10.48
ATOM   1257  CA  ILE A 178      32.203   4.246  14.783  1.00 15.25
ATOM   1258  C   ILE A 178      31.180   5.045  15.632  1.00 16.95
ATOM   1259  O   ILE A 178      31.092   4.774  16.851  1.00 22.68
ATOM   1260  CB  ILE A 178      33.338   5.121  14.357  1.00 25.11
ATOM   1261  CG1 ILE A 178      34.701   4.496  14.056  1.00 25.05
ATOM   1262  CG2 ILE A 178      33.599   6.205  15.392  1.00 27.60
ATOM   1263  CD1 ILE A 178      34.553   3.006  14.071  1.00 55.86
ATOM   1264  N   GLU A 179      30.218   5.799  15.178  1.00 16.34
ATOM   1265  CA  GLU A 179      29.290   6.610  15.985  1.00 16.94
ATOM   1266  C   GLU A 179      28.324   5.713  16.692  1.00 14.79
ATOM   1267  O   GLU A 179      27.683   6.012  17.716  1.00 19.20
ATOM   1268  CB  GLU A 179      28.555   7.637  15.169  1.00 21.16
ATOM   1269  CG  GLU A 179      28.790   7.283  13.691  1.00 50.37
ATOM   1270  CD  GLU A 179      29.933   7.701  12.851  1.00 61.82
ATOM   1271  OE1 GLU A 179      30.163   8.890  12.697  1.00 77.56
ATOM   1272  OE2 GLU A 179      30.627   6.854  12.309  1.00 75.83
ATOM   1273  N   ALA A 180      28.240   4.418  16.412  1.00  8.00
ATOM   1274  CA  ALA A 180      27.353   3.520  17.042  1.00 14.34
ATOM   1275  C   ALA A 180      28.048   2.991  18.280  1.00 19.53
ATOM   1276  O   ALA A 180      27.397   3.142  19.265  1.00 21.17
ATOM   1277  CB  ALA A 180      26.843   2.437  16.128  1.00 11.97
ATOM   1278  N   ARG A 181      29.317   2.547  18.287  1.00 21.89
ATOM   1279  CA  ARG A 181      29.992   1.982  19.398  1.00 16.48
ATOM   1280  C   ARG A 181      30.296   3.106  20.367  1.00 19.44
ATOM   1281  O   ARG A 181      30.243   3.104  21.639  1.00 28.53
ATOM   1282  CB  ARG A 181      31.310   1.408  19.143  1.00 12.43
ATOM   1283  CG  ARG A 181      31.954   0.432  20.052  1.00 45.44
ATOM   1284  CD  ARG A 181      32.596  -0.688  19.242  1.00 66.21
ATOM   1285  NE  ARG A 181      33.333  -0.030  18.164  1.00 85.83
ATOM   1286  CZ  ARG A 181      33.306  -0.321  16.895  1.00 91.35
ATOM   1287  NH1 ARG A 181      32.551  -1.320  16.530  1.00 96.98
ATOM   1288  NH2 ARG A 181      34.023   0.400  16.095  1.00 92.83
ATOM   1289  N   GLY A 182      30.387   4.262  19.847  1.00 13.94
ATOM   1290  CA  GLY A 182      30.553   5.404  20.728  1.00  7.40
ATOM   1291  C   GLY A 182      29.741   6.574  20.960  1.00  7.95
ATOM   1292  O   GLY A 182      29.171   6.512  22.083  1.00 12.73
ATOM   1293  N   GLU A 183      29.725   7.622  20.138  1.00  6.42
ATOM   1294  CA  GLU A 183      28.816   8.775  20.405  1.00 10.04
ATOM   1295  C   GLU A 183      27.421   8.369  20.645  1.00 14.41
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ATOM   1296  O   GLU A 183      26.846   8.530  21.749  1.00 15.43
ATOM   1297  CB  GLU A 183      29.053   9.791  19.402  1.00 21.24
ATOM   1298  CG  GLU A 183      28.079  10.638  18.725  1.00 62.21
ATOM   1299  CD  GLU A 183      28.248  12.103  19.141  1.00 81.34
ATOM   1300  OE1 GLU A 183      28.850  12.243  20.232  1.00 95.85
ATOM   1301  OE2 GLU A 183      27.791  13.027  18.430  1.00 90.85
ATOM   1302  N   ALA A 184      26.766   7.605  19.808  1.00 15.56
ATOM   1303  CA  ALA A 184      25.444   7.083  20.117  1.00 14.54
ATOM   1304  C   ALA A 184      25.549   6.382  21.464  1.00 13.62
ATOM   1305  O   ALA A 184      24.575   6.533  22.215  1.00 16.75
ATOM   1306  CB  ALA A 184      25.019   6.015  19.089  1.00  9.58
ATOM   1307  N   ALA A 185      26.428   5.396  21.774  1.00  9.42
ATOM   1308  CA  ALA A 185      26.219   4.677  23.031  1.00  7.48
ATOM   1309  C   ALA A 185      26.330   5.715  24.100  1.00 12.30
ATOM   1310  O   ALA A 185      25.761   5.503  25.179  1.00  9.50
ATOM   1311  CB  ALA A 185      27.138   3.475  23.260  1.00  4.60
ATOM   1312  N   ARG A 186      27.271   6.673  24.090  1.00 15.54
ATOM   1313  CA  ARG A 186      27.352   7.507  25.300  1.00 13.57
ATOM   1314  C   ARG A 186      26.085   8.286  25.561  1.00 11.49
ATOM   1315  O   ARG A 186      25.421   8.267  26.573  1.00  8.74
ATOM   1316  CB  ARG A 186      28.484   8.460  25.043  1.00 30.29
ATOM   1317  CG  ARG A 186      29.869   7.851  25.240  1.00 37.15
ATOM   1318  CD  ARG A 186      30.983   8.826  24.813  1.00 42.36
ATOM   1319  NE  ARG A 186      31.902   7.942  24.064  1.00 51.82
ATOM   1320  CZ  ARG A 186      32.324   8.346  22.870  1.00 50.20
ATOM   1321  NH1 ARG A 186      31.924   9.538  22.424  1.00 47.65
ATOM   1322  NH2 ARG A 186      33.115   7.476  22.318  1.00 39.90
ATOM   1323  N   PHE A 187      25.565   8.774  24.434  1.00  8.37
ATOM   1324  CA  PHE A 187      24.195   9.370  24.426  1.00 13.48
ATOM   1325  C   PHE A 187      23.187   8.476  25.182  1.00 15.92
ATOM   1326  O   PHE A 187      22.379   8.916  25.995  1.00 14.81
ATOM   1327  CB  PHE A 187      23.667   9.791  23.087  1.00 11.81
ATOM   1328  CG  PHE A 187      22.282  10.323  23.032  1.00 14.64
ATOM   1329  CD1 PHE A 187      21.984  11.586  23.391  1.00  8.47
ATOM   1330  CD2 PHE A 187      21.186   9.599  22.564  1.00 18.34
ATOM   1331  CE1 PHE A 187      20.698  12.134  23.353  1.00 12.89
ATOM   1332  CE2 PHE A 187      19.895  10.026  22.485  1.00 17.42
ATOM   1333  CZ  PHE A 187      19.661  11.322  22.924  1.00  3.70
ATOM   1334  N   LEU A 188      23.033   7.232  24.803  1.00 15.17
ATOM   1335  CA  LEU A 188      21.908   6.427  25.324  1.00 18.43
ATOM   1336  C   LEU A 188      22.207   6.221  26.775  1.00 19.67
ATOM   1337  O   LEU A 188      21.280   6.512  27.461  1.00 18.17
ATOM   1338  CB  LEU A 188      21.703   5.088  24.552  1.00 18.72
ATOM   1339  CG  LEU A 188      21.116   5.375  23.136  1.00  9.96
ATOM   1340  CD1 LEU A 188      20.950   4.066  22.601  1.00  7.86
ATOM   1341  CD2 LEU A 188      19.849   6.206  23.168  1.00  4.70
ATOM   1342  N   ARG A 189      23.333   5.805  27.230  1.00 17.48
ATOM   1343  CA  ARG A 189      23.798   5.812  28.547  1.00 18.41
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ATOM   1344  C   ARG A 189      23.353   7.039  29.321  1.00 16.87
ATOM   1345  O   ARG A 189      22.852   7.164  30.389  1.00 13.64
ATOM   1346  CB  ARG A 189      25.325   6.017  28.529  1.00 21.93
ATOM   1347  CG  ARG A 189      25.882   5.624  29.894  1.00 19.95
ATOM   1348  CD  ARG A 189      27.239   6.140  30.235  1.00 21.42
ATOM   1349  NE  ARG A 189      27.257   7.545  29.926  1.00 25.62
ATOM   1350  CZ  ARG A 189      28.491   7.983  29.699  1.00 29.22
ATOM   1351  NH1 ARG A 189      29.315   6.960  29.840  1.00 26.71
ATOM   1352  NH2 ARG A 189      28.780   9.210  29.383  1.00 33.27
ATOM   1353  N   ASP A 190      23.837   8.150  28.796  1.00 13.76
ATOM   1354  CA  ASP A 190      23.489   9.338  29.615  1.00 17.78
ATOM   1355  C   ASP A 190      22.008   9.364  29.711  1.00 16.79
ATOM   1356  O   ASP A 190      21.661   9.891  30.692  1.00 23.13
ATOM   1357  CB  ASP A 190      23.995  10.663  29.070  1.00 23.17
ATOM   1358  CG  ASP A 190      25.553  10.664  29.079  1.00 33.40
ATOM   1359  OD1 ASP A 190      26.250   9.836  29.761  1.00 22.68
ATOM   1360  OD2 ASP A 190      25.961  11.595  28.321  1.00 30.24
ATOM   1361  N   ARG A 191      21.156   9.128  28.781  1.00 21.61
ATOM   1362  CA  ARG A 191      19.707   9.265  28.849  1.00 20.99
ATOM   1363  C   ARG A 191      19.176   8.237  29.825  1.00 21.23
ATOM   1364  O   ARG A 191      18.327   8.515  30.651  1.00 20.98
ATOM   1365  CB  ARG A 191      19.014   9.214  27.450  1.00 19.76
ATOM   1366  CG  ARG A 191      19.605  10.282  26.521  1.00 27.49
ATOM   1367  CD  ARG A 191      18.848  11.594  26.689  1.00 36.68
ATOM   1368  NE  ARG A 191      17.559  11.023  27.144  1.00 60.89
ATOM   1369  CZ  ARG A 191      16.841  11.651  28.087  1.00 73.30
ATOM   1370  NH1 ARG A 191      17.404  12.780  28.496  1.00 76.65
ATOM   1371  NH2 ARG A 191      15.675  11.224  28.574  1.00 62.02
ATOM   1372  N   ILE A 192      19.734   7.037  29.885  1.00 21.02
ATOM   1373  CA  ILE A 192      19.500   6.080  30.913  1.00 21.92
ATOM   1374  C   ILE A 192      19.705   6.598  32.337  1.00 25.67
ATOM   1375  O   ILE A 192      19.145   6.053  33.263  1.00 27.95
ATOM   1376  CB  ILE A 192      20.289   4.775  30.750  1.00 24.23
ATOM   1377  CG1 ILE A 192      19.770   4.215  29.475  1.00 26.91
ATOM   1378  CG2 ILE A 192      19.923   3.983  31.951  1.00 15.15
ATOM   1379  CD1 ILE A 192      20.418   2.954  29.019  1.00 21.07
ATOM   1380  N   ARG A 193      20.535   7.574  32.629  1.00 28.72
ATOM   1381  CA  ARG A 193      20.800   8.068  33.963  1.00 33.95
ATOM   1382  C   ARG A 193      20.116   9.377  34.406  1.00 42.87
ATOM   1383  O   ARG A 193      20.479   9.267  35.618  1.00 48.19
ATOM   1384  CB  ARG A 193      22.298   8.179  34.167  1.00 34.19
ATOM   1385  CG  ARG A 193      23.096   6.896  34.100  1.00 39.38
ATOM   1386  CD  ARG A 193      24.590   7.213  34.133  1.00 65.92
ATOM   1387  NE  ARG A 193      25.339   5.973  34.003  1.00 81.05
ATOM   1388  CZ  ARG A 193      26.631   5.765  33.770  1.00 81.52
ATOM   1389  NH1 ARG A 193      27.441   6.816  33.647  1.00 80.92
ATOM   1390  NH2 ARG A 193      27.120   4.536  33.652  1.00 74.00
ATOM   1391  OT  ARG A 193      19.292  10.277  34.082  1.00 38.80
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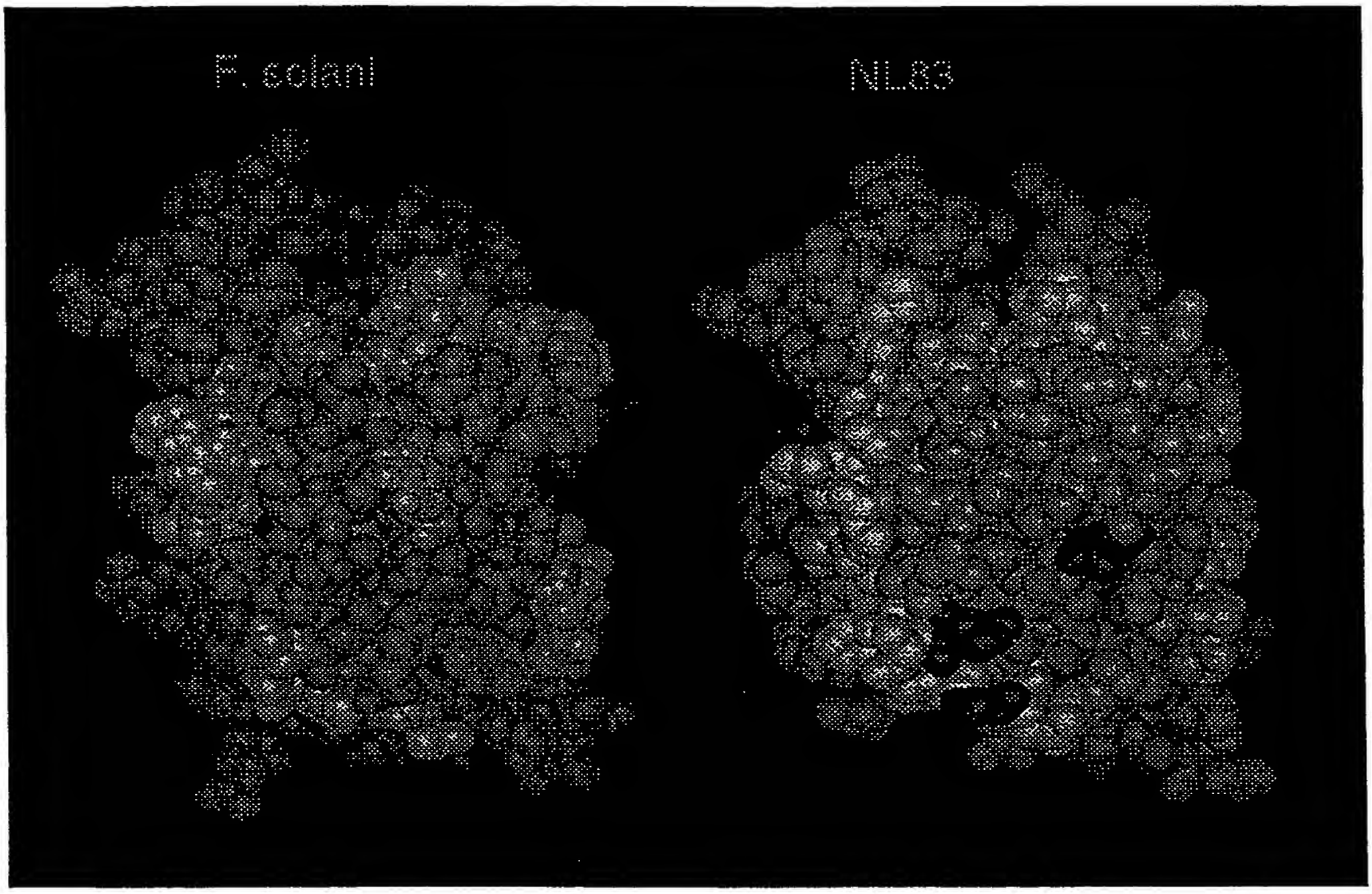

Fig. 2

3D structure of cutinases from *F. solani pisi* (left) and *H. insolens* (right)

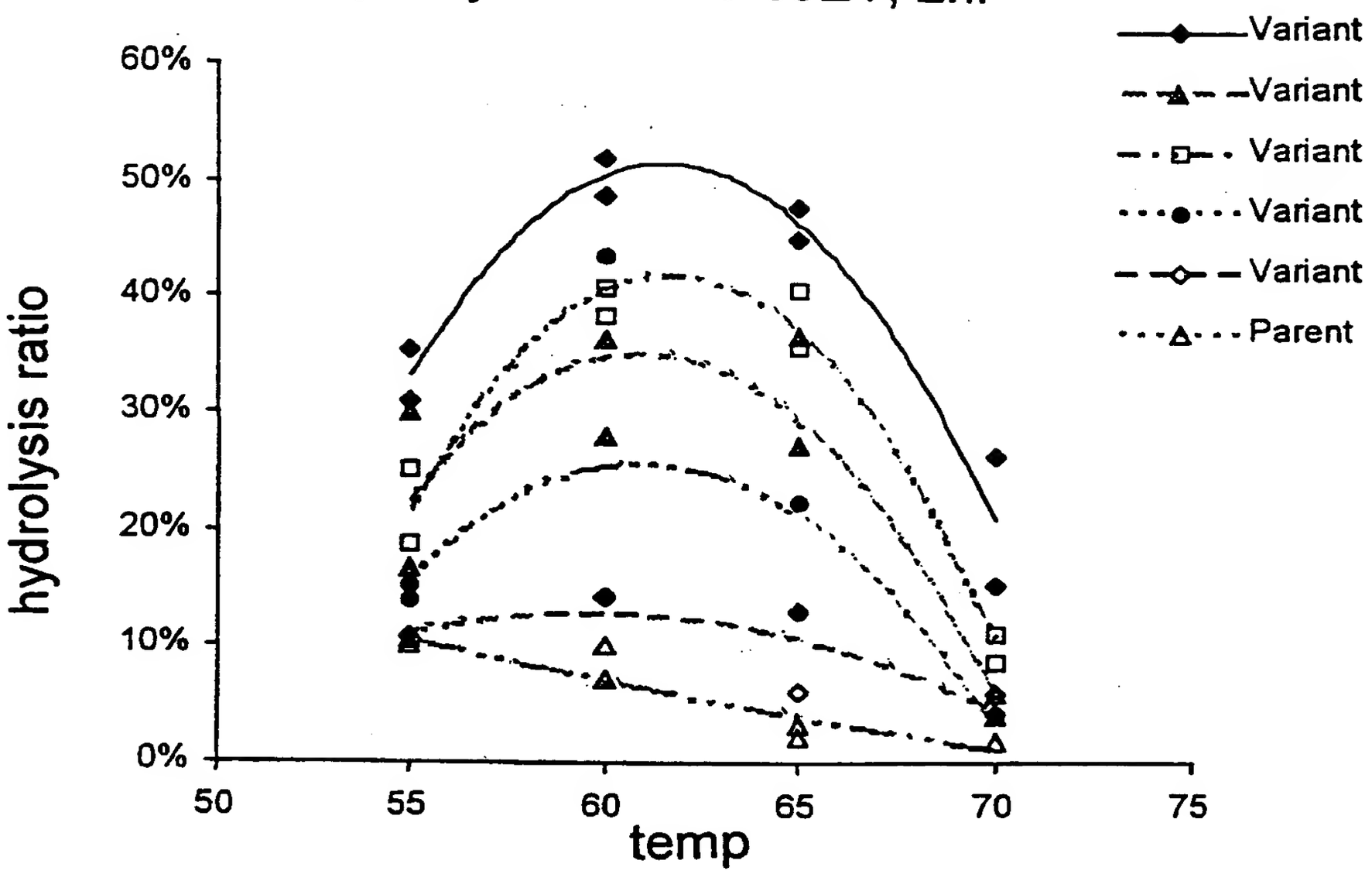

Fig. 3

Hydrolysis of solid c3ET, 2 hr

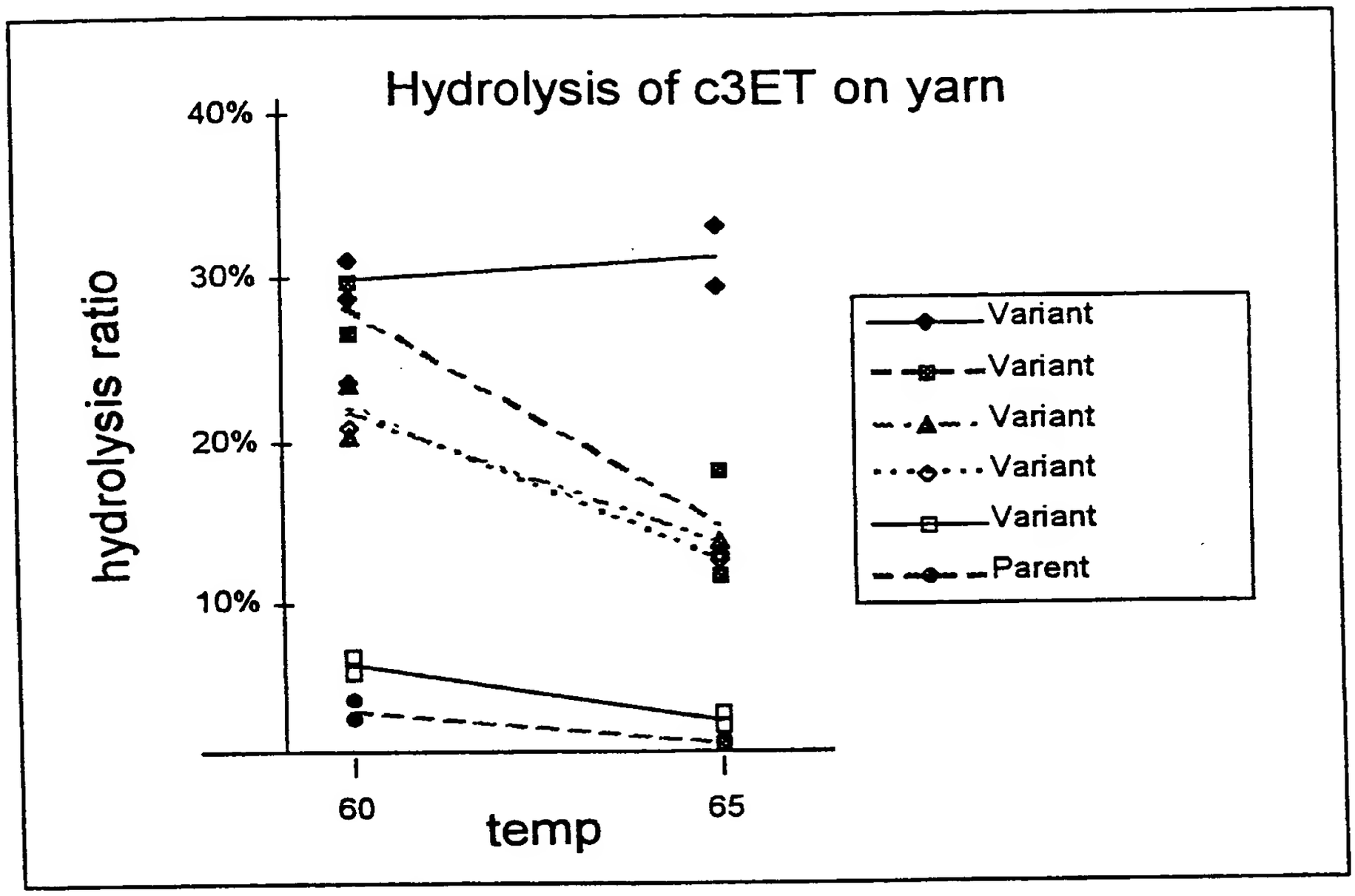

Fig. 4

Hydrolysis of c3ET on yarn, 17 hr

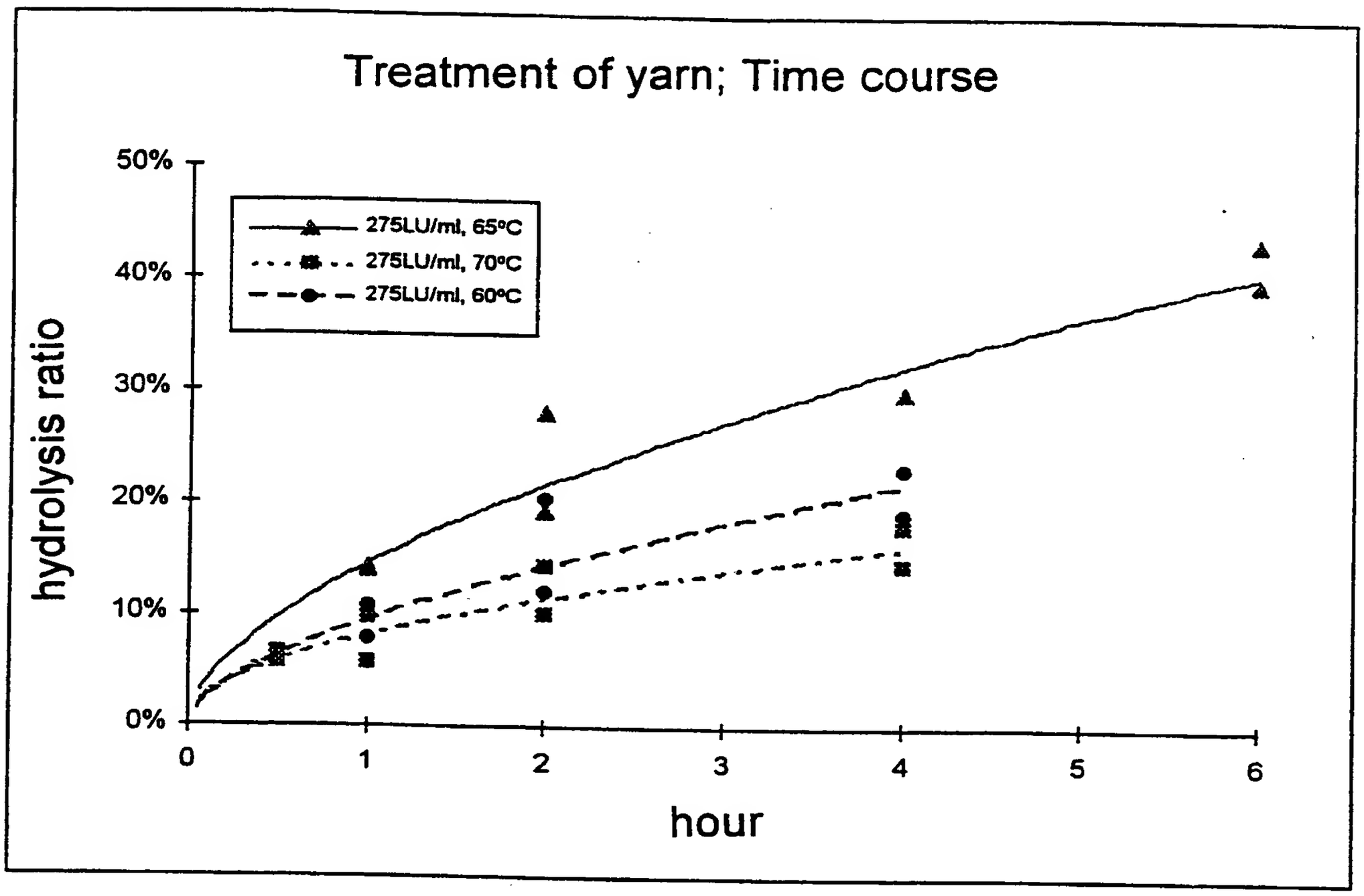

Fig. 5

Treatment of yarn; time course

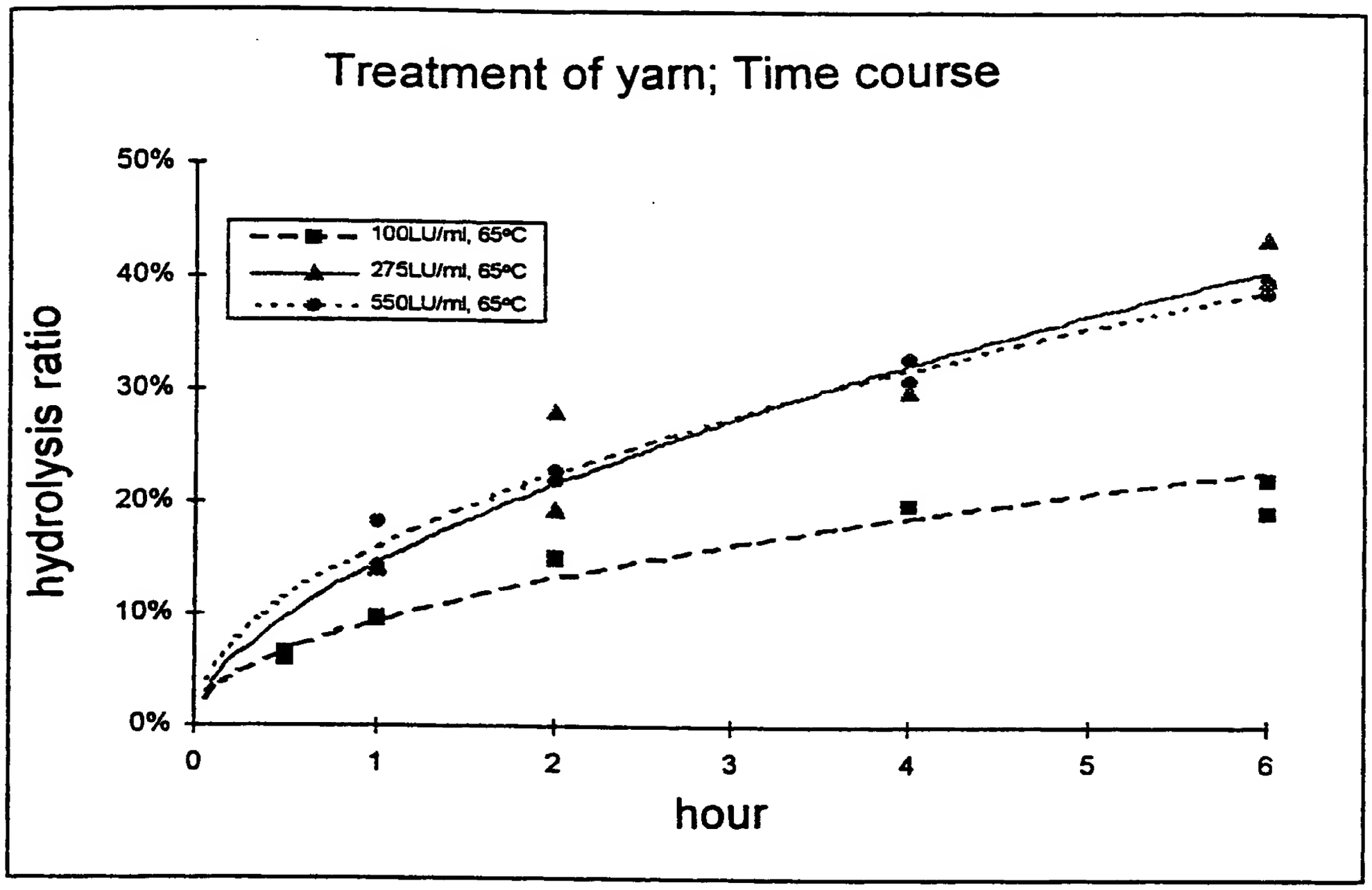

Fig. 6

Treatment of yarn; time course

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00678

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 9/18 // C11D 3/386, C08G 63/91
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9009446 A1 (PLANT GENETICS SYSTEMS, N.V.), 23 August 1990 (23.08.90), see page 1, lines 11-20, claims	1-32,34
X	WO 9414963 A1 (UNILEVER N.V.), 7 July 1994 (07.07.94), see claim 14	1-32,34
A	WO 9414964 A1 (UNILEVER N.V.), 7 July 1994 (07.07.94)	1-32,34
A	WO 9704078 A1 (NOVO NORDISK A/S), 6 February 1997 (06.02.97), see claim 51	1-32,34

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of th international search report
8 May 2000	11 -05- 2000
Name and mailing address of the ISA/ Swedish Patent Office	Authorized officer

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00678

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROTEINS: Structure, Function, and Genetics, Volume 26, 1996, Sonia Longhi et al, "Dynamics of Fusarium solani Cutinase Investigated Through Structural Comparison Among Different Crystal Forms of Its Variants" page 442 - page 458 -- --------	1-32,34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00678

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see next sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additi nal search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-32 and 34

Remark on Protest

☐ The additi nal search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00678

The invention claimed relates to two different inventions :

I. Claims 1-32 and 34 relate to cutinase variants and the use of these variants.
II. Claim 33 relates to a method for detecting cutinase activity in a sample.

Unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding "special technical feature" i.e. features that define a contribution which each of the inventions make over prior art. (See Annex B to administrative instructions and Rule 13.1).

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.
PCT/DK 99/00678

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9009446 A1	23/08/90	NONE	
WO 9414963 A1	07/07/94	AU 5699994 A	19/07/94
		CA 2150837 A	07/07/94
		CN 1090328 A	03/08/94
		CZ 9501578 A	13/12/95
		EP 0679188 A	02/11/95
		PL 309388 A	02/10/95
		SK 79595 A	08/11/95
		ZA 9309415 A	15/06/95
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		CN 1193346 A	16/09/98
		EP 0839186 A	06/05/98
		WO 9704079 A	06/02/97
		AU 6655196 A	12/03/97
		CN 1192780 A	09/09/98
		EP 0851913 A	08/07/98
		JP 11510699 T	21/09/99
		WO 9707202 A	27/02/97